

Project Number: KLB 0804

**"Bioreactor to Dynamically Condition Tissue-
Engineered Vasculature"**

**A Major Qualifying Report
Submitted to the Faculty of**

Worcester Polytechnic Institute

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

Submitted By:

Haseeb Ali

Jay Breindel

Samantha Bullock

Jesse Herrera

Date: April 30, 2009

Approved:

Professor Kristen Billiar (BME)

Professor Marsha Rolle (BME)

Professor Eric Overstrom (BBT)

Abstract

Cyclic loading is an important stimulus for creating mechanically stable tissue engineered blood vessels. The goal of this work is to create a device that dynamically conditions rings of vascular tissue created by cells in culture without the use of a scaffold. To create a high-throughput and accurate yet inexpensive device, a regulated pressure source and electronic valve controller were utilized to cyclically inflate up to 32 ring segments cultured on a series of silicone tubes encased in standard sterile conical tubes. This device will be used to determine the dynamic culture conditions that optimize engineered vessel growth and maturation in culture.

Executive Summary

I. INTRODUCTION

Coronary artery bypass surgery has become a widespread surgery in the United States, and since 2007 there have been more than 427,000 surgical procedures involving these small-caliber blood vessels.(American Heart Association, 2008) Coronary artery bypass surgery is necessary when a patient's artery has become blocked and blood needs to be rerouted around the blockage to keep the blood flowing and oxygenated. Currently, most of these surgeries are completed by harvesting small diameter blood vessels ($\leq 6\text{mm}$ diameter from elsewhere in a patient's body, such as the saphenous vein in the leg and internal mammary artery in the chest.(Parks, 2007)

Due to complications and limitations with using autologous blood vessels for transplantation in coronary artery bypass surgery, tissue engineering is being explored as an option for the source of vascular grafts. Patients lacking in suitable small-caliber vessels ($\leq 6\text{mm}$) for replacement in the coronary artery due to vascular disease, amputation, or previous harvest would especially benefit from a tissue engineering approach to address the need for alternatives. Although the ultimate goal is to generate a functional tissue replacement made entirely of living cells and their extracellular matrices, many different techniques have been utilized to produce a suitable vessel that can be used in coronary bypass surgeries. In our laboratory, we are currently developing vessel substitutes from cells in culture without the use of scaffolds. Due to the large number of factors involved, we require a high throughput means for optimizing media conditions and mechanical conditioning protocols.

A. Mechanical Conditioning

Mechanical conditioning, which involves imparting unidirectional or multidirectional forces on the cells being grown, has been identified as improving the mechanical properties of the tissue engineered constructs.(Seliktar et al., 2000) Advancement in the field of tissue engineered vasculature has recognized that such pre-conditioning specifically improves the ability of the cells and matrix to withstand the pressure exerted by the body's dynamic vascular system by inducing cell mediated remodeling of the collagen cell scaffold.(Seliktar et al.,2000)

B. Tissue Engineered Vascular Grafts (TEVG)

Ring-shaped segments of native blood vessels and tissue-engineered vascular grafts have been used as a model to test different experimental inputs such as mechanical conditioning and biological variables on tissue mechanical properties. The technique of growing rings allows the experimenter to avoid the rigor of growing entire engineered blood vessels and to thus efficiently determine optimal experimental inputs. The presence of properly structured and appropriate amounts of elastin and collagen, structural proteins found in the extracellular matrix, are considered a key outcome of successfully engineered vascular grafts.(Iwasaki et. al., 2008) Elastin is responsible for the property of the stretch and recoil of tissue, while collagen is responsible for ensuring mechanical tensile strength. Though these two components may oppose one another in their biomechanical function, they are required in congruence to maintain the viscoelastic properties of vasculature.(Vesely, 1998)

II. OBJECTIVES

The aim of this project was to design and create a device that will effectively mechanically condition tissue engineered vascular tissue rings. The following design objectives were identified:

- *Effective Device*: Must have the ability to accurately and reproducibly mechanically condition vascular rings.
- *High Throughput*: Must have the ability to condition many rings simultaneously, run multiple media conditions simultaneously, and/or change the longevity of the experiment and running time.
- *Mechanical Control*: Must have the ability to condition at multiple frequency levels and multiple strain levels.
- *Inexpensive*: Must be inexpensive to build and inexpensive to maintain.
- *Easy to Use*: Must be able to be operated by a trained scientist without difficulties.

The vascular rings will be placed on a device that will stretch them constantly or intermittently for a period of up to four weeks. The device was designed to allow simultaneous evaluation of multiple samples in parallel, so that media conditions may be evaluated to increase collagen and elastin synthesis to further improve tissue mechanical strength.

III. DESIGN

As seen in the diagram, next, four tissue-engineered vascular rings, XI, are placed on flexible, inflatable tubing.

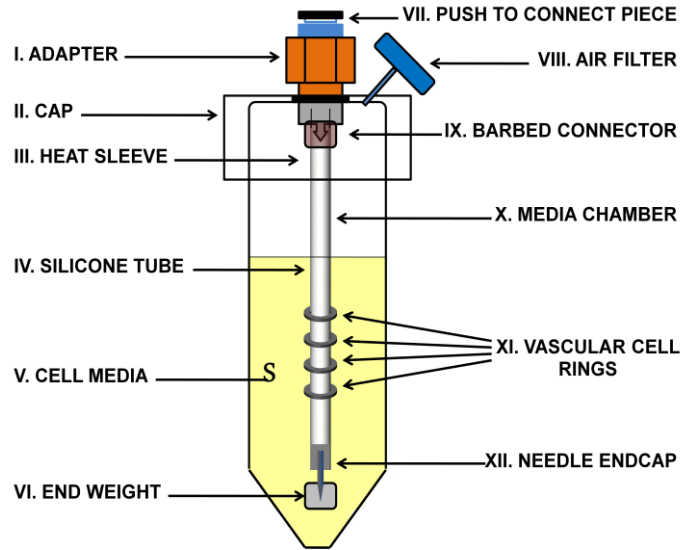


Figure i – Final Expandable Tube Design Schematic

To ensure non-cytotoxicity and the appropriate mechanical strength for dynamic loading, silicone, IV, is recognized and verified as a suitable option. The outside diameter of the silicone tubing is slightly smaller than the vascular rings. The flexible silicone tubing is connected to a threaded barb, IX, by a heat-sensitive shrinking sleeve, III. The threaded barb screws onto the cap, II, of a media chamber. A hole is drilled through the cap to ensure that the threaded barb fits tightly on the cap. An air pressure fitting, consisting of an adapter, I, and a push-to-connect piece, VII, screws on to the top of the barb on the opposite side of the cap. On the underside of the media chamber cap, between the cap and the barb connector's hex head, an o-ring, ensures that there is no leaking between the hole made in the cap and the outside environment. The barb and the silicone tube are housed inside the media chamber, X, with the appropriate media, V. A sterile air filter, VIII, attaches to the cap to allow the media to obtain oxygen. The needle endcap design, XII, explained in the Alternate Design section is used to seal the end of the silicone tube. Silicone glue is used as the adhesive between the silicone tube and the needle, as it verified to be strong enough to resist operating pressure and is

biocompatible. After the vascular rings are loaded, the needle endcap is inserted into an end weight, VI, which ensures the tube will hang vertically during experimentation. There are eight expandable tubing subsystems in the entire system as seen in the final computer model of the design shown next.

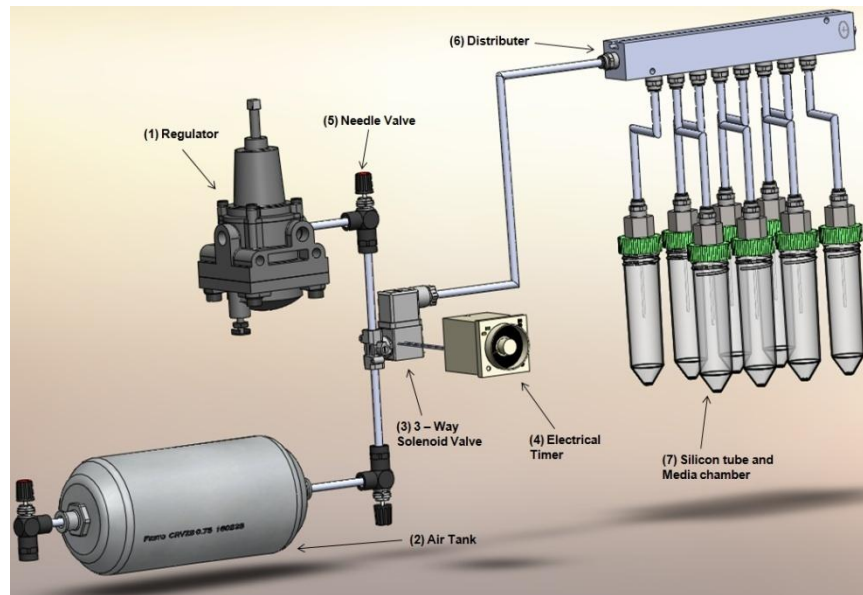


Figure ii – Computer Model of Final Design

The eight tubing units are attached to a manifold, or distributor (6). The numerous separate chambers allow for the high throughput required of the design. 50mL BD conical bottom polystyrene tubes are used as the media chambers. These centrifuge tubes are readily available in large quantities and easily replaced. Another benefit is the standard sizing which allows for all of the media chambers to fit tightly into the chamber caps. Furthermore, the caps are large enough to provide space for the barb fitting and a necessary sterile air filter.

A major aspect of the final design is the control of the load that is applied to the tissue-engineered vasculature. In the design, this is done through the use of a pressure control system which includes an air pressure source (from the wall), a pressure regulator

(1), an air volume tank (2), a three-way solenoid valve (3), an electrical timer (4), and needle valves (5). The pressure source is connected to the pressure regulator (1), via a standard T-valve. This regulator provides a constant high pressure value of 26psig which is necessary to obtain a 10% strain on the silicone tubing. The output of the regulator is connected to one of the two inputs of the three-way solenoid valve (3). The three-way solenoid valve is controlled by the electrical timer (4) to provide an output that switches back and forth between the high and low pressure inputs. A switch frequency of 0.5 Hz will be set on the timer, effectively controlling the solenoid valve to output the high and low pressures alternately at a frequency of 1Hz. The high-low pressure cycle will thus repeat every second. The air volume tank (2), with assistance of a needle valve, allows the pressure to leak back out of the system, providing a low pressure value. The needle valve on the air volume tank allows the low pressure to be calibrated to only reach the desired level of 4psig instead of emptying the system to 0psig. Two more needle valves (5) are placed between the regulator, air volume tank and the solenoid valve. The needle valves are adjusted and calibrated to ensure critical damping of the system, providing smooth transitions between the high and low pressures.

The output air pressure of the solenoid valve is distributed in parallel to each of the eight aforementioned silicone tubes via a manifold, or distributor (6) and equivalent length, polyurethane tube segments. All other components of the system described above are also connected to each other using polyurethane tube. A picture of the final product is shown next.

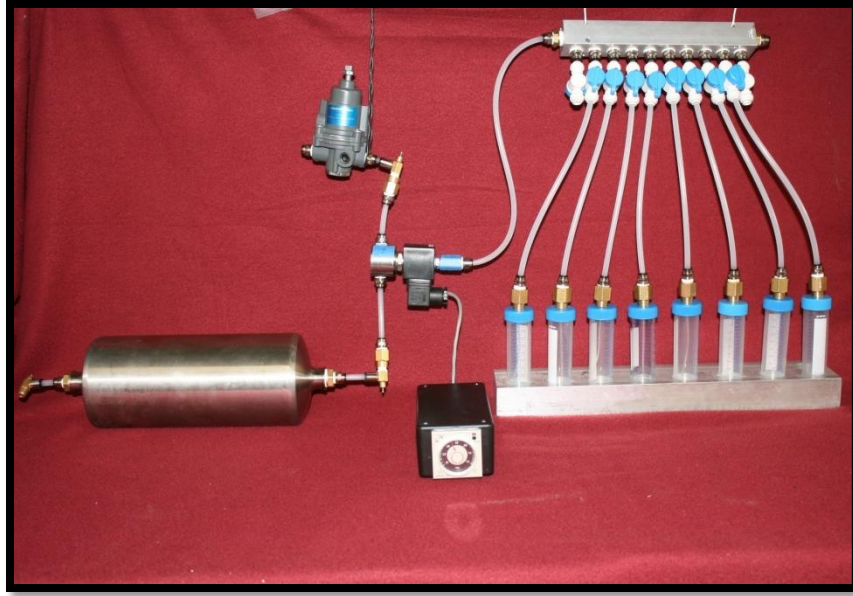


Figure iii – Final Bioreactor

This high-throughput device will be used to aid in determining the dynamic culture conditions that optimize engineered vessel growth and maturation in culture. It will ease and shorten the process of experimentation to allow scientists to study the effects different media and cell conditions have while vascular cell rings are undergoing mechanical conditioning.

Acknowledgements

This study is a result of collaboration between various labs at Worcester Polytechnic Institute. The team would like to thank several people for their assistance in this project. From the Rolle Lab at Gateway Park, WPI, Kshama Doshi, Tracy Gwyther, Jason Hu, and Darshan Parekh all helped with the verification and timely completion of this project. From the machine lab at Worcester Polytechnic Institute, Neil Whitehouse helped with the machining of important pieces of the design. From Minuteman Controls, Paul Gazaille helped extensively with identifying and ordering necessary parts.

Table of Contents

Abstract	ii
Executive Summary	iii
Acknowledgements	x
Table of Figures	xiii
Table of Tables.....	xiv
1.0 Introduction	1
2.0 Background.....	4
2.1 Tissue-Engineered Blood Vessels	6
2.2 Cell Derived Matrix.....	8
2.2.1 Collagen Production	9
2.3 Definition of Mechanical Conditioning.....	10
2.4 Previous Studies	11
2.4.1 General Cyclic Distension	11
2.4.2 Stretching on Membranes.....	12
2.4.3 Testing on Rabbit and Rat Blood Vessels	13
2.4.4 Cyclic Distension of Media Equivalents.....	13
3.0 Design Process.....	15
4.0 Alternative Designs.....	18
4.1 Stretch on Hooks	18
4.2 Stretch with Weight.....	22
4.3 Stretch on Cone	23
4.4 Stretch with Magnets	24
4.5 Stretch on Flexible Tube	24
4.6 Decision Matrix.....	28
4.7 Pressure System Tubing Connectors	29
4.8 Expandable Tubing Endcap.....	30
5.0 Verification of Subsystems and Discussion	34
5.1 Silicon Tubing.....	34
5.1.1 Initial Tubing Verification	34
5.1.2 Continuous Tubing Verification	37
5.2 Pressure Wave Calibration	40

5.3 Preliminary Assembly Verification	45
5.4 Method of Mounting Cell Rings.....	46
5.5 Verification of Media Chambers.....	47
6.0 Final Design.....	48
7.0 Conclusions and Recommendations	52
8.0 References	54
9.0 Appendix.....	57
Appendix A: Specifications for Vessel Ring Bioreactor.....	57
Appendix B: Objective Tree and Pairwise Comparisons.....	59
Appendix C: Waveforms from Pressure Calibration.....	61
Appendix D: Protocol for Vertically Testing Silicon Tubing	63
Appendix E: Protocol for Sub-Assembly Verification	64
Appendix F: Protocol for Verification of Regulators, Timer and Solenoid Valve.....	65
Appendix G: User Manual and Part List.....	66
Forward	66
Contents	67
Assembly.....	68
Preparation.....	78
Running the bioreactor.....	83
Complete Parts List.....	85

Table of Figures

Figure 1 - Coronary Artery Bypass Graft (© 1997- 2008 A.D.A.M., Inc.).....	4
Figure 2 - Rings as Tube Models.....	7
Figure 3 - Collagen	9
Figure 4 - Optimizing Effects of Dynamic Conditioning and Growth Factors	11
Figure 5 - Simple Hook Design	18
Figure 6 - Alternative Hook Orientations	19
Figure 7 – Alternative Hook Styles	21
Figure 8 - Spinning with Weight Design	22
Figure 9 - Rolling on Cone Design	23
Figure 10 - Stretching with Magnets Design	24
Figure 11 - Extending Tube Design.....	25
Figure 12 - Single Row of Vertical Tubes (Flat and Slanted Bottoms).....	26
Figure 13 - Two Rows of Vertical Tubes	26
Figure 14 - Horizontal Tube Arrangements.....	27
Figure 15 - Two Media Chamber Design	28
Figure 16 - Individual Media Chamber Design	28
Figure 17 - After Loading Rings Endcap Designs.....	30
Figure 18 - Before Loading Rings Endcap Designs	32
Figure 19 - Marking Locations on Silicone Tube.....	36
Figure 20 - Strain at Top Mark on Silicone Tube.....	36
Figure 21 - Strain Values at Varying Tube Locations	38
Figure 22 - Outside Diameter Measurements at Varying Tube Locations	39
Figure 23 - Dynamic Pressure System.....	41
Figure 24 - Original Pressure Control System Schematic	42
Figure 25 - Single Regulator Pressure Control System Schematic.....	43
Figure 26 - Single Regulator Pressure Waveform	43
Figure 27 - Air Volume Tank Pressure Control System Schematic	44
Figure 28 - Critically Dampened System Pressure Waveform.....	45
Figure 29 - Needle Endcap Schematic, Actual, and Implementation	47
Figure 30 - Final Expandable Tube System Schematic	48
Figure 31 - Final Design Computer Model.....	49
Figure 32 - Final Product	51
Figure 33 - Objective Tree.....	59
Figure 34 - System Critically Dampened at the High and Under-dampened at the Low .	61
Figure 35 - Completely Under-dampened System.....	61
Figure 36 - System Critically Dampened High and Over-dampened Low.....	62
Figure 37 - Critically Dampened System.....	62

Table of Tables

Table 1 - Hook Style Comparisons	22
Table 2 – Design Decision Matrix	29
Table 3 - Main Objectives Pairwise Comparison	59
Table 4 - Mechanical Control Pairwise Comparison	60
Table 5 - High Throughput Pairwise Comparison	60

1.0 Introduction

Coronary artery bypass surgery has become a widespread surgery in the United States, and since 2007 there have been more than 427,000 surgical procedures involving these small-caliber blood vessels (American Heart Association, 2008). Coronary artery bypass surgery is necessary when a patient's artery has become blocked and blood needs to be rerouted around the blockage to keep the blood flowing and oxygenated (Schaffer, 2007). Currently, most of these surgeries are completed by taking larger-caliber blood vessels from elsewhere in a patient's body, such as the internal mammary arteries and saphenous veins, and using them to reroute blood around the blocked artery (Isenberg et. al., 2006).

Due to complications and limitations with using autologous blood vessels for transplantation in coronary artery bypass surgery, tissue engineering is being explored as an option for the source of vascular grafts. The benefit of a tissue-engineered method becomes apparent when recognizing the need for blood vessels that are small in diameter ($\leq 6\text{mm}$), similar to the ones they are replacing. Patients lacking suitable small-caliber vessels for replacement in the coronary artery due to vascular disease, amputation, or previous harvest (Isenberg et. al., 2006) would especially benefit from a tissue engineering approach to address the need for alternatives.

Many different techniques have been experimented with to produce a suitable vessel through tissue engineering techniques that can be used in coronary bypass surgeries. Although the ultimate goal is to generate a functional tissue replacement made entirely of living cells and their extracellular matrices, the process in which these vessels are constructed can vary. Five approaches that address the complicated architecture and

unique mechanical properties of the vascular wall are endothelial cell (EC)-seeded synthetic grafts, cells suspended in fibrin or collagen-gels, biodegradable synthetic polymer-based constructs (with or without seeding), cell sheet-based blood vessels, and decellularized tissue approaches to vascular graft engineering (Seliktar et. al., 2000). Though these novel procedures have made significant strides in creating fully functional engineered vasculature, significant limitations still prevent their clinical use. These include limitations in having the cells adhere to the synthetic polymers and ensuring the mechanical integrity of the constructs in vivo (Seliktar et. al., 2000). Furthermore, the goal of developing a fully functional vascular graft will likely require knowledge gained from each one of these approaches.

Different techniques result in different mechanical and biological properties with compromises between the efficacies of different parameters used to determine suitable and effective vasculature for transplantation. Mechanical conditioning, which involves imparting unidirectional or multidirectional forces on the cells being grown, has been identified as improving the mechanical properties of the tissue-engineered constructs (Isenberg et. al., 2006). Advancement in the field of tissue-engineered vasculature has recognized that such pre-conditioning specifically improves the cells' ability to withstand the pressure exerted by the body's dynamic vascular system; for example, by inducing cell-mediated remodeling of the collagen cell scaffold. (Isenberg et. al., 2006) The results of combining both cell and media types and the process of mechanical conditioning are very limited. A system needs to be developed to allow for comparisons of biological factors while cells are simultaneously being dynamically stimulated.

The aim of this project is to design and create a model system that will effectively mechanically condition tissue-engineered cellular rings. The cellular rings will be fabricated independently, and then placed on a device that will stretch them constantly or intermittently for a period of up to four weeks. This biological goal of this work is to find the specific combination of cell media type that induces collagen synthesis that, when grown under dynamic mechanical conditions, will create strong engineered tissue rings.

2.0 Background

Coronary heart disease is the leading cause of death in men and women in the United States (Michaels, 2002). Consequently, coronary artery bypass graft (CABG) surgery is one of the most common operations performed throughout the world (Eagle, 2004). CABG surgeries are mainly conducted because plaque forms in the coronary arteries resulting in a process known as atherosclerosis, or hardening of the arterial walls (Parmet, 2008). This plaque blocks the arteries, decreasing blood flow and oxygen to the heart, causing angina and myocardial infarction (American Heart Association, 2008). CABG operations take blood vessels, usually from elsewhere in the patient's body, and use those vessels to bypass the blockage (Isenberg et. al., 2006). Preferably, small caliber blood vessels like the saphenous vein, radial arteries and internal thoracic arteries should be used since they are close in size and function to the blocked vessel. Figure 1 shows a cartoon of a CABG procedure.

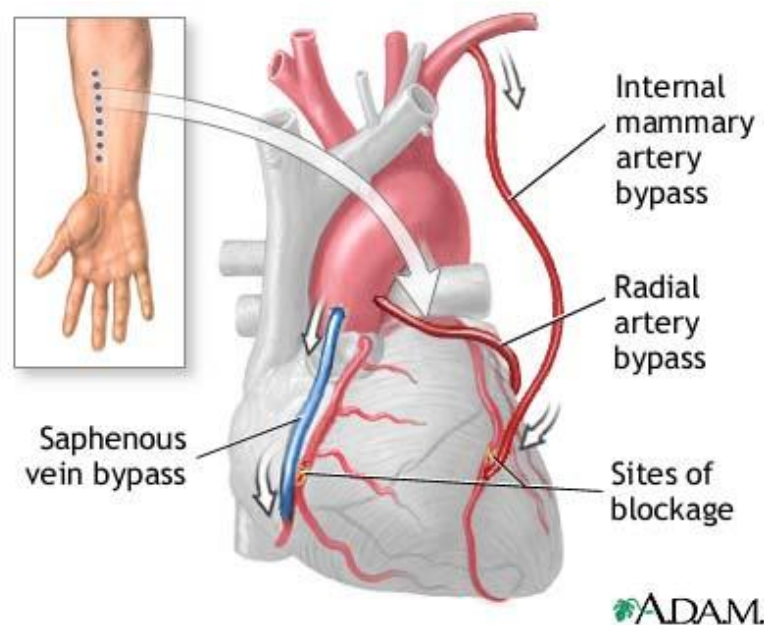


Figure 1 - Coronary Artery Bypass Graft (© 1997- 2008 A.D.A.M., Inc.)

Functional problems arise when small caliber (<6mm) are unavailable (Isenberg et. al., 2006). Although synthetic grafts have been explored as an alternative, the low blood flow environment in these arteries is associated with increased thrombogenicity and intimal hyperplasia, leading to synthetic graft failure (Shastri, 2004). Tissue engineering has emerged as a promising alternative that may limit the effects of thrombogenesis due to their biological origin. An ideal bioengineered graft would be mechanically strong and pliable, non-immunogenic, non-thrombogenic, easy to handle, and cheap to produce (Shastri, 2004).

One of the major differences between synthetic, tissue-engineered and autologous conduits is the presence in the latter of a living functional layer of endothelial cells resting on a metabolically active, smooth muscle cell media. Current synthetic grafts, lack these properties and are unable to ‘heal’ completely in humans, failing to achieve the necessary tissue ingrowth that would encourage coverage of the luminal surface by endothelial cells (Isenberg et. al, 2006). These blood vessels, whether prosthetic or tissue-engineered must be able to withstand the pressures of the human body and they must possess the appropriate mechanical and biological properties needed to make them indistinguishable from the native cells and vessels (Isenberg et. al, 2006). It is widely held that 3 components are necessary for these criteria to be met: (1) a biocompatible component with high tensile strength to provide mechanical support (collagen fibers or their analogue); (2) a biocompatible elastic component to provide recoil and prevent aneurysm formation (elastin fibers and their analogue); and (3) a non-activated, confluent endothelium to prevent thrombosis (Isenberg and Tranquillo, 2003). The different

techniques for engineering vasculature address these criteria, each with their own advantages and disadvantages.

2.1 Tissue-Engineered Blood Vessels

There are many different ways that blood vessels can be constructed using tissue-engineered techniques. The ultimate goal is to create a blood vessel made entirely of cells and their extracellular matrices. Many of these methods use scaffolds for the cells to aggregate around (Isenberg et al., 2006). One method starts with biodegradable scaffolds on which cells are seeded and allowed to produce their extracellular matrix (Isenberg et. al., 2006). Once visible tubes are formed the cells are washed away and only the matrices are left, leaving the tubes acellular (Schaffer, 2007). In theory these acellular tubes are then to be implanted into the patient and repopulated by the body's own cells (Schaffer, 2007). Biodegradable polymer scaffolds and biopolymer gels are also methods used to generate tubular vessels (Syedain et. al., 2008). The cells are seeded onto a multitude of different biodegradable synthetic polymer scaffolds or gels and once the cells have formed into vascular tubes the polymer dissolves leaving behind a hollow vascular tube (Isenberg et. al., 2006, Syedain et. al., 2008).

Another method to produce blood vessels is growing the cells into two dimensional sheets and then rolling them into the shape of a tube (L'Heureux et. al., 2007). The sheets are made completely from cells and no synthetic or exogenous materials are used (L'Heureux et. al., 2007). The tubes are created from multiple layers of cells that fuse together to create a tube that will withstand the pressures of the human body (Isenberg et. al., 2006). The cell sheets are grown on culture plate and then wrapped around a porous tubular mandrel and an outer layer of cells is wrapped around

that to construct the adventitia, which is the outermost connective tissue of the vessel (L'Heureux et. al., 2007).

While all of the above methods ultimately end in the production of a tissue-engineered blood vessel they all have major problems that need to be addressed. All of the methods involving scaffolds as well as those vessels made from sheets have a lack of compliance that keeps the vessels from performing to their full potential (Isenberg et. al., 2006). The vessels made from decellularized tissues have experienced reduced tensile strength and a lack of compliance (Isenberg et. al., 2006). A lack of compliance implies high stiffness. The vessels made on biodegradable scaffolds have experienced premature weakening of the tissue (Isenberg et. al., 2006). The lack of compliance prevents the engineered vessels from maintaining their structural integrity, as such vessels undergo unrecoverable plastic deformation. Better methods for constructing tissue-engineered blood vessels have greatly improved the mechanical properties of these constructs.

Rather than generate an entire blood vessel and then take sections of it to mechanically test, some researchers are growing cellular rings that have the same properties as an entire blood vessel and are testing them as model representation of small diameter vasculature (Isenberg and Tranquillo, 2003). This representation is shown in the schematic below.

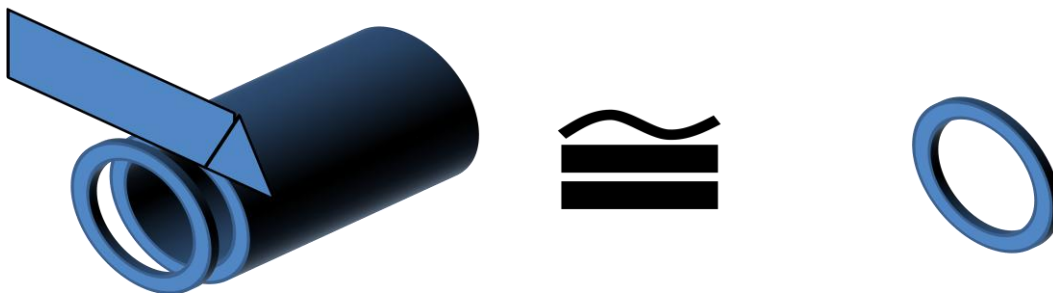


Figure 2 - Rings as Tube Models

This technique is both easy to replicate and time efficient. Since investigators have not yet been able to develop a fully functional bioartificial artery (BAA) that meets the requirements for mechanical strength and biological functionality (Isenberg and Tranquillo, 2003), the idea of creating rings that mimic BAAs is an approach that will allow researchers to have a biological model through which to study and improve the functionality of tissue-engineered vasculature.

2.2 Cell Derived Matrix

Patients that have undergone myocardial injury have scar tissue from local remodeling that inhibits the vessels from functioning properly and they must have vascular grafts implanted to continue smooth functioning of the heart and vessels (Bunda et al, 2007). In previous studies, vascular tissues have been made from cells, so therefore the cellular matrix must be adjusted to withstand the pressures of the heart and blood vessels (Bunda et al, 2007).

The cells that these vessels are made of become an important decision because they have to be compatible with the rest of the system as well as be able to function like cells in a blood vessel. Smooth muscle cells and dermal fibroblasts are two types of cells that are very common among previous studies (Bunda et al, 2007).

The cell matrix is also a very important part of the vascular tissue. Different types of cells synthesize different matrix components and it is crucial to have the appropriate composition of cellular matrix components to create an ideal vascular tissue graft.

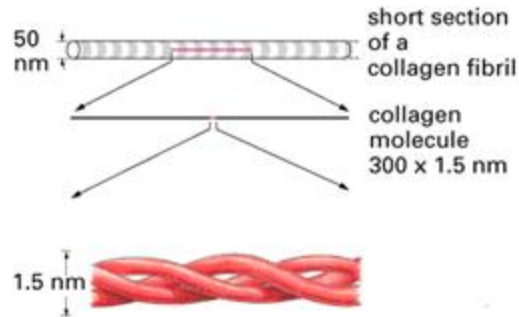


Figure 3 - Collagen

2.2.1 Collagen Production

Collagen fibers are composed of three extended protein chains that wrap around one another to form a triple helix (Alberts et al, 2004). Collagen molecules are cross-linked in the ECM to form collagen fibrils which form collagen fibers, as depicted in Figure 3 (Berwal and Novakofski, 1999). Collagen is a glycoprotein which has intermolecular cross-links, which impart tensile strength (Berwal and Novakofski, 1999). Collagen is needed in the ECM for tensile strength.

Hydroxyproline is a modified amino acid exclusive to collagen and ascorbate has been shown to contribute to the metabolic processes which produce hydroxyproline, leading to collagen production (Davidson et al, 1997). The amount of ascorbate added to the cells in culture has a direct affect on collagen production; the more ascorbate that is added, the more collagen is produced (Davidson et al, 1997).

Ascorbate is a cofactor in the enzyme activity of hydroxylase which stimulates collagen production and is needed for collagen to attain its triple helix formation under physiological temperatures (Davidson et al, 1997). Based on the data collected by Davidson et al, ascorbate added to human dermal-fibroblasts at varying concentrations (0, 0.5, 5, and 25 $\mu\text{g/mL}$) resulted in a dose dependent increase in collagen synthesis.

2.3 Definition of Mechanical Conditioning

An efficient arterial replacement graft needs to not only function biologically like a natural vessel but also must function mechanically like a natural vessel. Mechanically, a vessel needs to be able to withstand shear stress, pressure and stretching due to blood pressure, and longitudinal tension along the vessel (Isenberg et al, 2006). There have been different methods established to replicate natural vessel properties, such as varying cell culture conditions and cell types, but a promising, recently developed method is mechanical conditioning, or dynamic stimulation (Isenberg et al, 2006, Sung In Jeong et al., 2005, Cummings et al., 2004, Isenberg and Tranquillo, 2003). Several studies have shown that *in vitro*-cultured arterial cells that are exposed to mechanical signals, (like those experienced by natural vessels) develop many attributes of efficient vascular grafts for *in vivo* usage. Such attributes include enhanced mechanical strength, collagen production, cell alignment, and regulated phenotype of vascular smooth muscle cells (Isenberg et al, 2006, Sung In Jeong et al., 2005, Cummings et al., 2004, Isenberg and Tranquillo, 2003). It has been shown that mechanical stretching promotes the expression of type I and III collagens, fibronectin, and tenascin-C in cultured ligament fibroblasts (Sahoo et. al., 2007). Another study indicated that human bone marrow mesenchymal stem cells (hBMMSC) - even in the absence of biochemical regulators - could be induced to differentiate into ligament-like fibroblast by the application of physiologically relevant cyclic strains (Vunjak-Novakovic et. al., 2004). Therefore, mechanical conditioning has become a primary strategy for inducing tissue growth and maturation *in vitro* (Freed et. al., 2000). An assortment of bioreactors have been manufactured to expose cell structures to mechanical stimulation (Isenberg et al, 2006). However, these bioreactors

have never been used to test the effects of different culture conditions, a goal that the team hopes the design accomplishes. These experimental parameters can be growth factors, such as cell and media type, that contribute to changes in the architecture of the extracellular matrix. As seen in Figure 4, the team plans to use this bioreactor to combine both methods of cyclic distention and growth factors to obtain experimental results. The problem is illustrated in the schematic below.

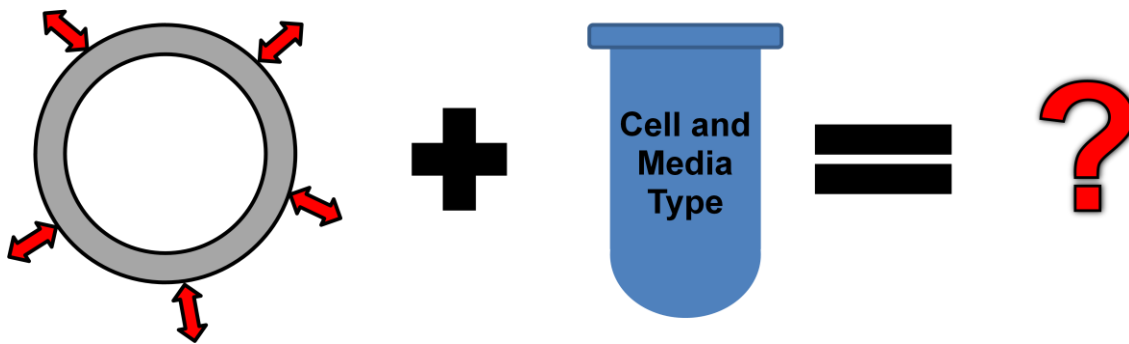


Figure 4 - Optimizing Effects of Dynamic Conditioning and Growth Factors

2.4 Previous Studies

There have been many bioreactor configurations and mechanical conditioning techniques developed by researchers to further understand the effects of mechanical conditioning on the properties of arterial replacement graft tissue.

2.4.1 General Cyclic Distension

Cyclic distention has become the primary method of conditioning vascular smooth muscle cell media equivalents (Syedain et. al., 2008). Cyclic distension can be achieved by successive increase and decrease of the outer diameter of a balloon being inflated and deflated. In the case of the cyclic distension of vasculature, the balloon is replaced with a small diameter elastic tube, around which the rings or entire vessels can be loaded and similarly inflated and deflated. Within cyclic distension, there have been

many slightly different techniques used. Recent research from Sung In Jeong *et al* shows one example, where rabbit aortic smooth muscle cells were cultured in a pulsatile perfusion bioreactor for 8 weeks. The cell culture was allowed to grow circumferentially around a flexible, cylindrical scaffold and it was exposed to a pulsatile strain of 5% of initial radius at a rate of 1 Hz. As a control, vascular smooth muscle cells were cultured on a similar scaffold for the same duration without cyclic distention. Pulsatile strain was found to enhance the cells' proliferation and collagen production. In addition, a significant cell alignment in a direction radial to the distending direction was observed whereas the control, statically-cultured cells, not as uniformly aligned.

2.4.2 Stretching on Membranes

Similarly, Dartsch and Hammele grew arterial smooth muscle cells from rabbit aortic media on lyophilized and collagen-coated silicone membranes. These membranes were then subjected to anywhere from 2% to 20% cyclic and directional stretches and relaxations at a frequency of 1Hz. The experiment used unstretched membranes as a control and after several days, the degree of orientation of the cells was shown to increase directly with the increase in stress applied. In a similar experiment by Wilson et al., cells were grown on flexible silicone elastomer plates. The cells were then subjected to a 1Hz cyclic strain by a vacuum underneath the plates. A forty-eight hour period of cyclic strain showed an increase in population growth of the cells and an increase in mRNA level for PDGF, both of which are positive cellular attributes. Ching-Hsin Ku *et al.* observed similar results using aortic valve interstitial cells from pigs.

2.4.3 Testing on Rabbit and Rat Blood Vessels

Kolpakov et al. used rabbit pulmonary arteries to determine the effects of applied stretching. After four days of stretching, protein synthesis within the smooth muscle cells increased, as did cell replication and the amount of pro-collagen type I-positive cells. Similarly, Liu et al. showed the results of applying a tensile strain to rat blood vessels. Strain on the vessels, tested after ten, twenty, and thirty days of applied strain, resulted in better alignment of the smooth muscle cells.

2.4.4 Cyclic Distension of Media Equivalents

Girton et al. applied a one-dimensional strain of up to 50% on a smooth muscle tissue equivalent. The strain was determined by measuring the displacement of polystyrene beads dispersed within the collagen fibrils. After twenty-four hours the cells once again showed more alignment than before the strain had been applied. An experiment by Kim et al. showed that short term cyclic strain resulted in increased proliferation of smooth muscle cells and increased expression of collagen and elastin. Long term results showed better cell alignment, up regulated elastin and collagen gene expression and an overall increase in the general mechanical properties of elastic modulus and tensile strength of the tissues. Lee et al. showed an increase of core proteins in a related study. O'Callaghan and Williams used a "Flexercell Stress Unit" by Flexcell Corp. to stretch human vascular smooth muscle cells ten to sixteen percent. After five days the cells showed a 48% increase in fibronectin and a 50% increase in collagen. Isenberg and Tranquillo investigated key parameters involved in long-term cyclic distension as they pertain to the development of collagen-based media equivalents (MEs). They first cultured smooth muscle cells from rat aortas. They used highly compacted,

cross linked constructs to avoid complicated issues of creep and transient alignment which they recognized in other cyclic distention experiments. The prepared MEs were ring-shaped and were placed over distensible mandrels. The mandrels were cyclically pressurized to induce radial strain to the rings. The rings were strained at various amplitudes, frequencies, pulse shapes and durations. As a control, a number of rings were grown under static conditions. After two weeks of cyclic distention, the mechanical properties of the MEs were not significantly different than those in the control. But, after five weeks, MEs that were cyclically strained had significant increases in strength and stiffness.

3.0 Design Process

In order to develop a design, it was necessary to start by interviewing the client. This allowed us to begin to understand what the final designed product must be able to do. With the information from the interview, a client statement was made. With this client statement, the project group finalized a list of objectives for the project.

- *Effective Machine*: Must have the ability to accurately mechanically condition vascular cell rings.
- *High Throughput*: Must have the ability, if desired, to condition many rings simultaneously, run multiple media conditions simultaneously, and/or vary culture duration.
- *Mechanical Control*: Must have the ability to condition at multiple frequency levels and multiple strain levels.
- *Inexpensive*: Must be inexpensive to build and/or inexpensive to maintain.
- *Easy to Use*: Must be able to be operated by a trained researcher without difficulties.

There are also many functions that are required for the design. The machine must be able to hold at least thirty vascular cell rings. It must be able to accurately, cyclically stretch these rings ten percent at a frequency of 1 Hz. The machine must also be sterile and all components which are in direct or indirect contact with the cells need to be sterilizable. There also has to be an easy method to feed the cells.

Once the client statement was established, the importance of each objective for the design and the sub-objectives for each main objective needed to be ranked. To

develop an understanding of what the sub-objectives were, an objective tree was created, as seen in Figure 33 in Appendix B. Once the objectives and sub-objectives were determined, pair-wise comparisons were used to allow the client to rank which objectives and sub-objectives were more important when designing the machine. The pair-wise comparisons are shown in Tables 3 through 5 in Appendix B.

After reviewing the pair-wise comparisons, and summing up the scores for each objective, it was determined that the most important objective was the ability to have a high throughput system. As seen in the objective tree, high throughput means having the ability to stretch multiple rings at once, run multiple media conditions simultaneously, and run for a time period of between one to four weeks. The second most important objective was for it to be effective. Ranked after ease of use, the design is required to be easy to use and handle by a trained operator. The client provided constraints of stretching the cell rings ten percent at a frequency of 1 Hz. The cost of the product was also constrained to one thousand dollars. Cost and mechanical control are thus two properties that must further be taken into the consideration of the design of the bioreactor.

Under the main objective of mechanical control there are sub-objectives as seen in the objective tree. Although the main objective of mechanical control is not one of the most important objectives for the design, the sub-objectives must still be ranked by the client. As seen in Table 3, being able to control the strain and having an accurate strain on the cell rings are the most important sub-objectives for mechanical control.

The main objective of high throughput also has sub-objectives. It is very important to take these sub-objectives into consideration because the main objective of high throughput was the most important objective to the client. Table 5 shows the pair-

wise comparison for the sub-objectives under high throughput. From this table it can be concluded that being able to have a large number of samples is the most important sub-objective. Another important sub-objective is having the ability to run more than one media condition simultaneously. An obvious design requirement not listed is the need for the device to be biocompatible (non-cytotoxic).

Overall, the interviews and objective comparisons established that the focus of the design would revolve around having an effective device that allowed for high throughput; many rings and many conditions. If possible, the less important objectives of variable mechanical controls and having an easy to use system would be taken into consideration

4.0 Alternative Designs

The most important aspect of the bioreactor design was the ability to radially stretch and relax vascular cell rings. To develop this subsystem of the machine, many possible methods of stretching were designed, developed, and compared. In this section, the alternative designs will be discussed and compared.

4.1 Stretch on Hooks

The method of stretching rings on hooks was thoroughly investigated. Many different variations of stretching on hooks were devised and considered. The first variation that was brainstormed was a simple hook system as seen in Figure 5. The hooks in this system move back and forth, stretching and relaxing the ring that rests on them.

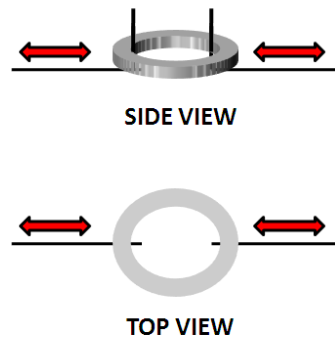


Figure 5 - Simple Hook Design

The main problem with this system is the point force that occurs where the hook makes contact with the ring. This is a disadvantage for the desired radial stretch. From this system, additional variations were created for both hook style and hook arrangement. Hook styles were developed to decrease the point force that would be applied on the cell

rings. Hook arrangement were varied to allow for higher throughput in the system. The stretching subsystem needs to allow for many rings to be distended simultaneously.

To design for the major objective of high throughput, different hook orientations were considered. The design also was focused on being compact to allow the system to fit inside of an incubator, allowing for constant optimal cell conditions. The four orientations are shown next in Figure 6. In this figure, Orientation 1 consists of two lines of hooks. These lines slide back and forth allowing for stretching and relaxing of the cell rings. Orientation 2 consists of two large hooks on which the cell rings are stacked for stretching. Orientation 3 consists of two sets of hooks, situated in a “pliers like” fashion. When each arm is rotated back and forth, the rings are pulled and relaxed, applying the necessary cyclic stretch. Lastly, the fourth orientation is similar to orientation 3. Orientation 4, however, consists of more hook arms that are rotated back and forth. Another difference is the fact that each ring is positioned on one moving hook and one fixed hook instead of two moving hooks.

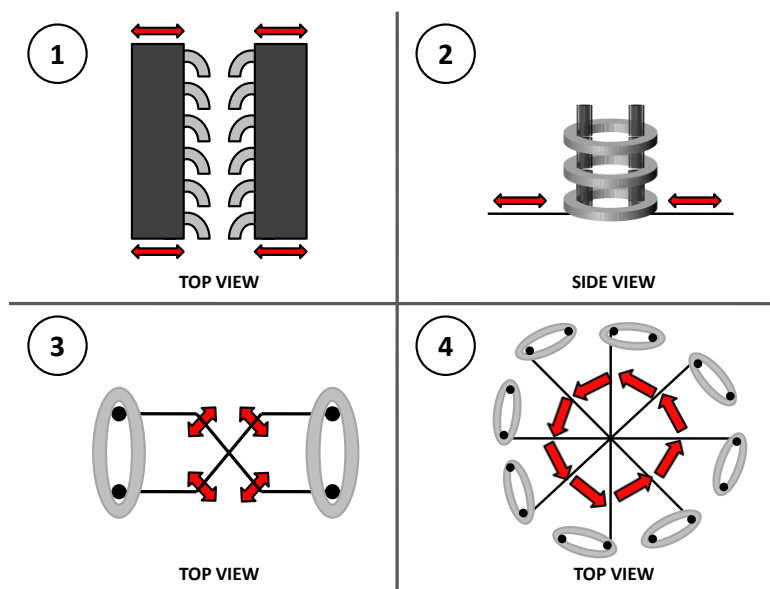


Figure 6 - Alternative Hook Orientations

Each hook orientation has benefits and problems. For Orientation 1, the rings would easily be loaded in a high throughput manor. The first orientation also would allow for a compact size to be used within an incubator. The disadvantage of this orientation is a lack of consistency while stretching numerous rings. It would be difficult to ensure that every hook was positioned in the exact same way for all of the cell rings. The second orientation requires many rings to be stacked upon each other and stretched on the same hooks. This would allow for consistency in the stretching of the rings and high throughput. The downfall of this design is the possible damage that would occur when rings are stacked upon each other.

Orientation 3 would once again allow for consistent stretching of the cell rings. This method, however, has much less high throughput and it would be difficult to arrange the hook arms in a compact fashion. Finally, Orientation 4 would be a higher throughput way of arranging the arms in Orientation 3. This orientation would be difficult to set up inside an incubator, however. The best orientation design seems to be the movable lines of hooks in Orientation 1.

To eliminate the point force caused by the simple hook design discussed previously, alternative hook styles were designed. The first style is, as previously mentioned, thin and straight hooks. This style would allow for easy ring loading but could potentially damage the ring with the point forces that are applied. From this initial style, four more styles were developed. These styles are shown next in Figure 7. Style 1 is a thick hook style, similar to the thin hook design. Style 2 is a “U” hook design. Style

3 is a thick hook style with an additional bend as seen in Figure 7. Style 4 is flat hook style.

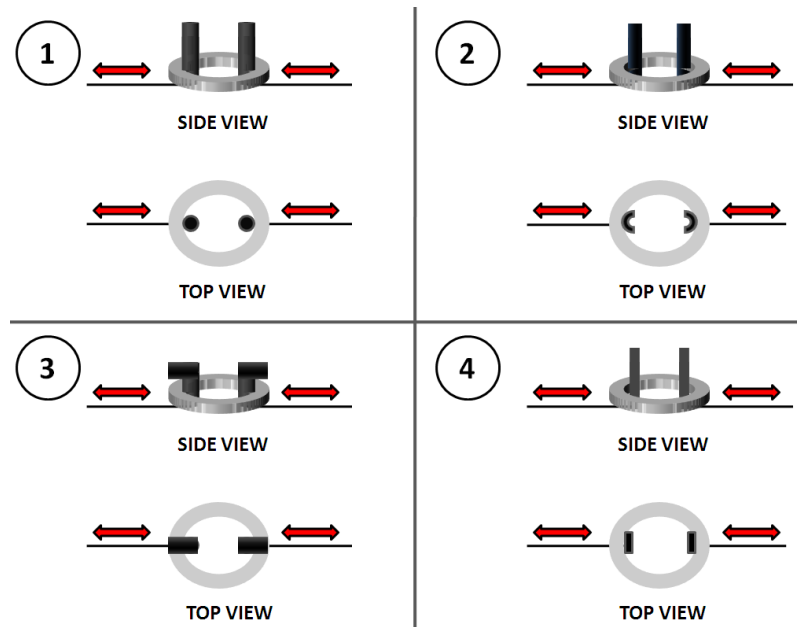


Figure 7 – Alternative Hook Styles

Style 1 allows for a smoother contact between the hooks and the ring, but still does not apply a completely uniform stretch. The rings can be loaded easily on this style of hook. Style 2 also allows for easy ring loading onto the hooks. This style was developed to obtain a smoother contact point between the hooks and the rings. Once again, however, the stretch is not completely uniform. Style 3 would protect against losing the ring during the dynamic stretching experiments. The additional bend would secure the ring into place for the entire experiment. This style, however, could pose a potential inconvenience when loading the rings due to the need to extra bend. Finally, Style 4 would allow for easy ring loading, but would not provide a smooth contact between the ring and the hooks. The comparisons of the five hook styles, the original hook style and the four styles listed in Figure 7, are listed in Table 1.

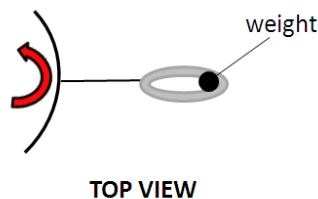
Table 1 - Hook Style Comparisons

Style	Ease of Loading	Smooth Contact	Uniform Stretch	Secure Hold
Thin Simple Hook	✓	-	-	-
Thick Simple Hook	✓	✓	-	-
“U” Hook	✓	✓	-	-
Bent Hook	-	✓	-	✓
Flat Hook	✓	-	-	-

The most important column in the hook style comparison table is the uniform stretching. Since none of the styles allow for a completely uniform stretch around the circumference of the ring, other stretching methods needed to be developed.

4.2 Stretch with Weight

Another option for ring stretching that was investigated was distending the rings using an attached weight and a spinning disk. The design for this method is seen in Figure 8, next.

**Figure 8 - Spinning with Weight Design**

In this design, the cell ring undergoes a stretch by spinning the ring with a weight attached to the outer edge of the ring. The inertia of the weight causes it to pull from the

center of the spinning disk, resulting in a distention of the cell ring. This method, however, has many more disadvantages than benefits.

First of all, it would be very difficult to obtain a precise stretch when using this method. The exact weight and speed needed would be very hard to determine. Secondly, the desire for the bioreactor is a cyclic distension, meaning the ring must cycle back and forth between being stretched and relaxed. The spinning with weight design would only provide an extension of the ring and it would be very challenging to establish a slow spinning speed that would allow the ring to relax. Finally, this method would not allow for easy high throughput. Since high throughput and accuracy are very important to the final design, this method was rejected.

4.3 Stretch on Cone

Another method that was quickly rejected was rolling the cell rings up and down a cone. This method would stretch the rings as they moved farther down the cone and relax the rings as they moved back to the top of the cone. The design of this method is shown next in Figure 9.

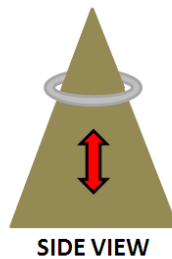


Figure 9 - Rolling on Cone Design

Once again, the rolling on a cone method does not allow for the desired high throughput. There is also a high probability that the rings will be damaged during the

rolling of the ring in this process. Due to the lack of high throughput and precise control in this method, further methods needed to be developed.

4.4 Stretch with Magnets

One promising concept the group came up with was to stretch the cell rings by using magnets that are moved in and out by magnetic attraction. If done correctly, this process could allow for both precision and accuracy. The method would also allow for high throughput. The design for stretching with magnets is shown next in Figure 10.

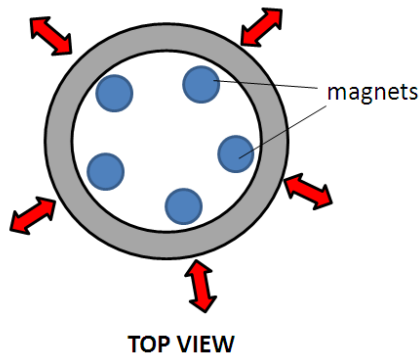


Figure 10 - Stretching with Magnets Design

The magnets would provide a safe and more uniform stretch on the vascular cell rings. Although this method seems very promising for future work, it was decided that stretching with magnets was too expensive and not feasible for the team to complete. Another, less technical approach needed to be taken.

4.5 Stretch on Flexible Tube

The approach of stretching the cell rings on a flexible tube rapidly became the most promising method for the team to investigate. The method would not only allow for precise stretching of the cell rings but would also allow for clear high throughput methods. The simple expandable tubing design is shown next in Figure 11.

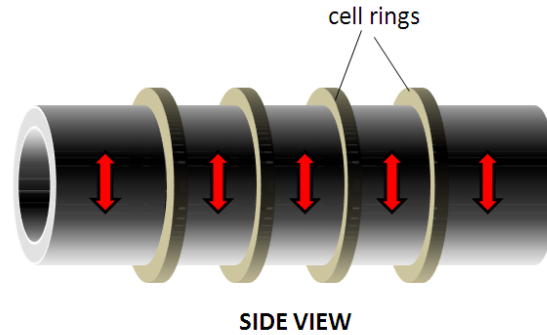


Figure 11 - Extending Tube Design

For this design, cell rings will be loaded around an expandable tube. The tube will then be pressurized to inflate and deflate the tube a controlled amount. The rings will in turn be stretched and relaxed on the outside of the inflating tube. This method would provide high throughput by allowing multiple rings to be distended on each tube. Furthermore, precise positions on each tube can be calculated to ensure that each ring is stretched the desired amount.

It was decided that many separate expandable tubes would be used in the final bioreactor. For this idea to work, many tube arrangements were considered. The final arrangement needed to be compact and allow for easy use within an incubator. The first two basic designs are shown in Figure 12, next. The first design is a single row of tubes within a media chamber, while the second arrangement is the same design but it includes an additional slanted bottom. This slanted bottom was designed to ease the necessary process of changing the cell media.

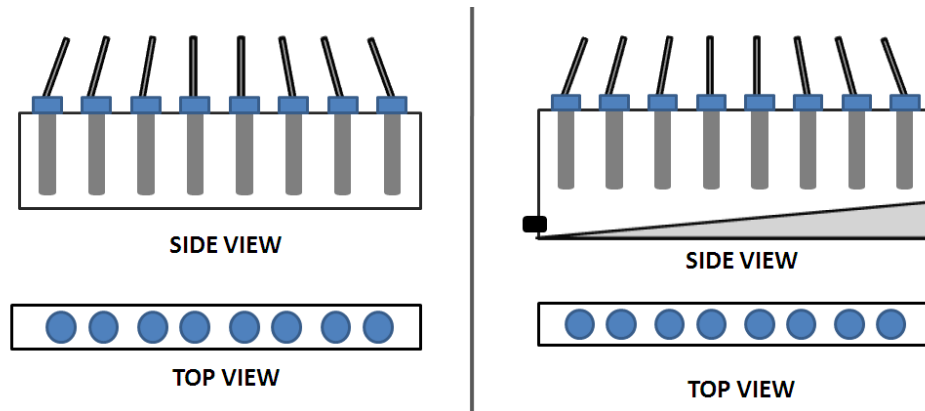


Figure 12 - Single Row of Vertical Tubes (Flat and Slanted Bottoms)

Another similar arrangement allows for a more compact set of tubes. In this version, there are two rows of vertical hanging tubes. This arrangement is shown in Figure 13, next. An additional arrangement containing three rows of vertical tubes was determined to be too difficult for set up and use.

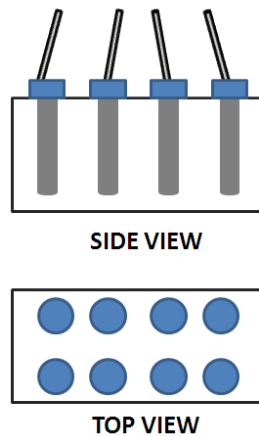


Figure 13 - Two Rows of Vertical Tubes

Two more arrangements include single and double rows of horizontal tubes. The benefit of these styles is the need for much less media to cover the entire tube. The problem with these tube arrangements is the chance that flexible tubes will not remain completely horizontal when inserted into the media. Many holder designs were created

to help with the flexible tube problem, but it was decided that the overall arrangement of horizontal tubes was inferior to vertical tubes.

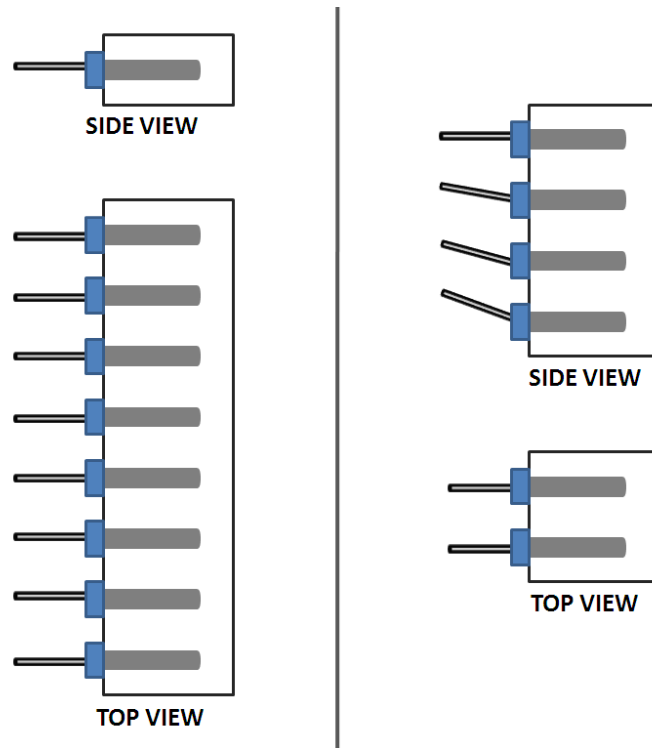


Figure 14 - Horizontal Tube Arrangements

Another advantage of the extending tube design is the ability to test multiple cell and media types simultaneously. This capability is achieved by using multiple media chambers, each having one or more individual expandable tubes. To accomplish the multiple media chamber style, additional media chamber arrangements were designed and compared.

There were two main designs for the multiple chamber arrangements. The first of these styles has two media chambers, each with multiple expandable tubes. This arrangement is shown in Figure 15. This approach would allow two media conditions to be compared simultaneously.

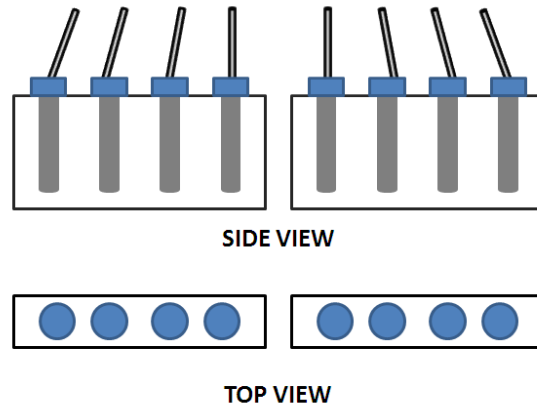


Figure 15 - Two Media Chamber Design

The final design included a separate media chamber for each individual expandable tube. This style would allow for many different media conditions to be tested simultaneously on many different cell rings. This method allows for the greatest amount of high throughput and mechanical control. The design for this arrangement is shown in Figure 16.

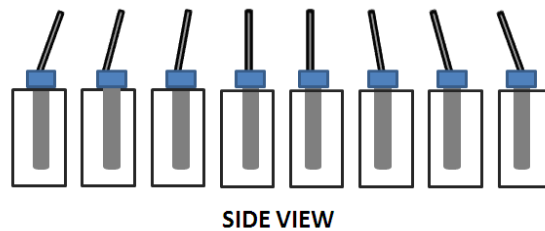


Figure 16 - Individual Media Chamber Design

4.6 Decision Matrix

All of the alternative designs were compared to one another with regards to the original design objectives. The comparisons are shown in the decision matrix. The designs were ranked from one to five, where five is the best for each category.

Table 2 – Design Decision Matrix

Design Alternatives	High Throughput	Mechanical Control	Inexpensive	Easy to Use	Total
Hook System	4	2	5	5	16
Spinning Weight	2	1	2	1	6
Cone System	1	3	3	3	10
Magnetic Strain	3	5	1	2	11
Inflatable Tube System	5	4	4	4	17

It can be seen in this decision matrix that the best design for the team's objectives is the inflatable tube system. Although some of the other designs seemed promising, they did not completely meet the goals for the bioreactor.

4.7 Pressure System Tubing Connectors

Once the final design concept was reached, each of the sub-systems of the machine required further research and designing. A pressure subsystem was required to provide an internal air pressure to the expandable tubes. Pulsatile fluid flow was considered but deemed too difficult to achieve for the bioreactor. The first parts that were investigated were the tubing connectors for the entire pressure system. There were many available connector types, such as barb fittings, push-to-connect fittings and quick clamp fittings. The requirement for the rigid tube connectors was that they be air tight, in order to avoid any leakage, and easy to connect and disconnect, in order to allow easy media replacements in the system. The project group determined that the push-to-connect fittings were the best option for this subsystem due to the high pressure ratings and ease of connection and disconnection that is associated with this type of connector.

A tube connector is also required to connect the expandable tubing to the air system in the media chambers. The barb fittings were decided upon to connect the flexible tubing to the air system since the tubes would be able to expand and contract around the barb to provide a tight seal. A biocompatible shrinking heat sleeve would also be wrapped around the barb to ensure a tight seal.

4.8 Expandable Tubing Endcap

An important aspect of the expandable tubing design is the open bottom end of the expandable tube. An airtight endcap needed to be designed. Since the rings needed to be loaded onto the tube at some point of the system set up, there were two main categories for the endcap designs; plug after loading the rings and plug before loading the rings. Multiple designs for each category were created and compared.

There were four main designs for the plug after loading category. They are all shown next in Figure 17. All four designs are too bulky and inhibited loading rings after they are in place.

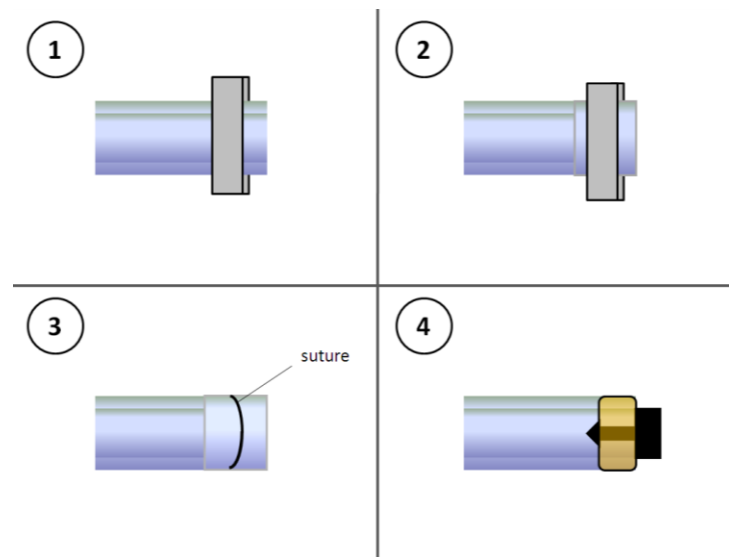


Figure 17 - After Loading Rings Endcap Designs

The first endcap design shown in Figure 17 is a simple crimp at the open end of the tube. This design is very similar to the second design, which requires the tube to be folded over upon itself and then crimped. These two designs would most likely provide a reliable airtight seal. The second design with the fold in the tube would most likely be more reliable with the seal. The third design involves, once again, a fold in the end of the tube. In this case, however, the tube is sealed by tying a suture. The suture tied fold would be less reliable and much more difficult to apply. The final design for this category is very similar to the connection at the other end of the expandable tube. A solid barbed connector would be inserted into the open end of the tube and then sealed with a biocompatible heat sleeve. This endcap would provide a very reliable seal and would not be overly difficult to set up.

The problem with all four of the designs where the rings were loaded before the endcap was attached, is that the rings would be at risk for damage. The cell rings could be harmed while sitting on the tube during the endcap attachment. The team decided that limiting the ring exposure would be preferred, requiring endcap designs that were applied before the rings were loaded.

For the next set of endcaps, the designs needed to be small enough to allow the rings to be slipped onto the tube over the endcap. There were once again four different designs to be compared. The four designs are shown in Figure 18.

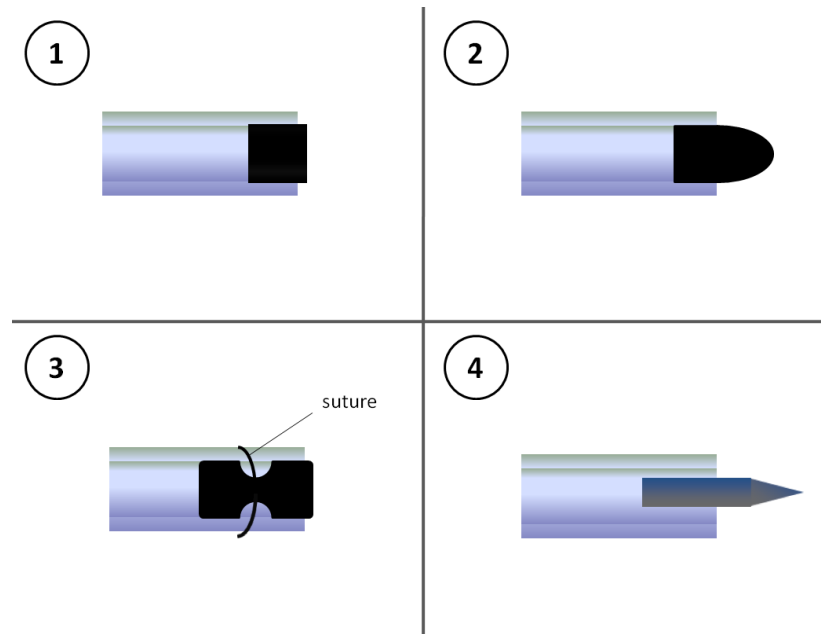


Figure 18 - Before Loading Rings Endcap Designs

The first of the smaller endcap designs is a small cylindrical piece that is glued into the open end of the expandable tube. This endcap would provide a reliable airtight seal and would not be hard to insert. The second design is very similar, but involves a round piece that is glued into the open end of the tube. This round edge was added to ease the process of ring loading. It would be easier to guide the ring onto the tube with a rounded cap instead of a flat ended cap. The third design is very similar to the other designs as well. The difference for this endcap is instead of glue, a suture is used to tie the endcap around a slot, holding it in place. It would be much more difficult to attach this endcap. In addition, there would be much less reliability in the seal provided by this endcap. The final design involves a small needle glued into the open end of the expandable tube. This option was designed to fulfill both the needs of a reliable seal and ease of ring loading. The glue would provide an air tight seal. The needle will be used in ring loading by allowing the point of the needle to be inserted into an agarose well containing the cell rings.(Bullock, 2009) For functional reasons, the chosen endcap was

the needle endcap. The benefits for both a reliable seal and an easier ring loading method were the deciding factors for this endcap design.

5.0 Verification of Subsystems and Discussion

The bioreactor was constructed one subsystem at a time. This allowed each subsystem to be tested individually to ensure that they met their performance requirements. A complete list of performance specifications is found in Appendix A.

5.1 Silicon Tubing

It was determined that the material in contact with the cell rings would need to be strictly biocompatible, non-degradable and chemically inert. After researching different materials, silicon and latex were singled out. It was determined that silicon tubing would be the best option. Silicon tubing is readily commercially available, relatively inexpensive and completely non-cytotoxic. Latex, on the other hand, is cheaper than silicon tubing, but can be cytotoxic and thus not suitable for the project at hand. After obtaining the silicon tube, the elastic properties needed to be determined so as to insure that the tubing would expand to the appropriate strain within tolerable pressure.

A barbed connector would provide a connection between the silicone tubing and the air pressure system. In order to provide a seal that was able to withstand up to 30psig, the tubing connection required more than just fitting the expandable tube over the barb. The group tested heat-sensitive sleeves, glue, and sutures to solve this problem. Neither the glue nor the suture seals were able to provide enough strength to hold the flexible tubing onto the connector. The heat-sensitive sleeves shrink when heat is applied, contracting them around the tube and the barb, providing an extremely tight seal.

5.1.1 Initial Tubing Verification

For an effective design, the rings needed to be strained at least 10% of their original diameter. Since the intended method of this was to pressurize a silicon tube

around which 4 rings would fit, an accurate pressure- strain relationship of the tube needed to be obtained over a substantial length of tube. Thus, to verify the use of silicon tubing, the effects of pressure on the diameter/distention of the tube needed to be studied. Pressure was increased from 0 psig to 30 psig, in increments of 2psig. Pressure was then decreased from 30 psig to 0 psig. Measurements of the outside diameter were made using a DVT vision sensor. Strain measurements were made from these measurements. A pressure to strain correlation was thus made. The detailed experimental procedure is included in Appendix D. After it was determined that the silicone tube was capable of withstanding a 10% strain, the team needed to determine at what pressure the silicone would inflate to this value. Also required was the amount of pressure the silicone tube would require to inflate to the inside diameter of the vascular cell rings, which are grown on 2 mm posts, as the silicon tube has a slightly smaller outer diameter of 1.96 mm. Shown below is the pressure-strain curve for the top mark. As there is some variation between trials, further testing was conducted to determine a pressure value needed to strain the silicone 10%.



Figure 19 - Marking Locations on Silicone Tube

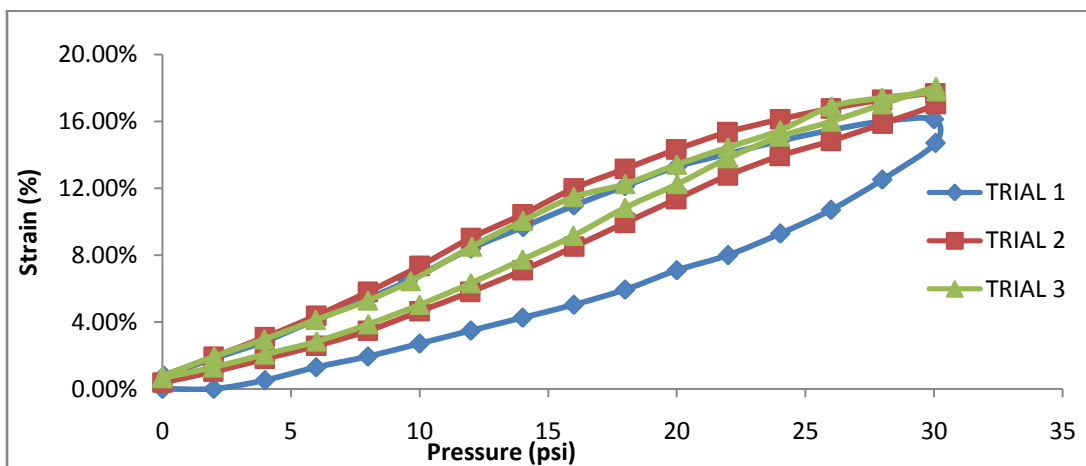


Figure 20 - Strain at Top Mark on Silicone Tube

Due to the silicone tube bursting after only the second round of testing, it was determined that a smaller diameter barb was needed. Stretching the silicone tube around the barb compromised the mechanical integrity of the silicone tube, which resulted in a burst near the top of the tube.

5.1.2 Continuous Tubing Verification

The silicone tube was dynamically tested for 5 days at a frequency of 1 Hz, at a high pressure of 18 psig, which corresponded to approximately a 10% obtained strain from initial testing. The test was conducted in media chambers filled with water, to simulate actual experimental conditions. This testing also experimented with the new end-needle design described earlier. After 5 days of testing, it was observed that extensive bubbling had occurred on the silicone tube. The tube had also started to float and adhered to the side of the media chamber.

The problem regarding bubbling was successfully fixed by adding water to the inside of the tube, which prevents air from leaking out of the silicone and creating the bubbles. This was confirmed after running the system for another 2 days.

After the silicone tube had been cycled, the silicone tube was measured using the same approach as used to measure strain in the first round of testing described above. In this case, three marks were made near the bottom of the tube and measured successively. It was determined that on average, a 10% strain was achieved at 24psig. This is the value that a pressure regulator can be set at while cycling the vascular rings. This value is slightly higher than what was originally determined, possibly due to the affect of filling the inside of the silicone tube with water. To stretch the 0.77in (1.96mm) tube to 2mm (the diameter of the rings), 4 psig of pressure was needed. Here, no significant correlation

between the amount of strain at a given pressure and the elevation could be made. This experiment also verified the use of silicone glue as no leaking from the silicon glue was observed after 5 days, and then subsequently after 2 more days. To guarantee that no leaking occurs, the proper procedure for sealing the end of the silicone tube needs to be followed. This procedure is outlined in the User Manual. Shown below is the strain at the three different marks on the bottom of the silicone tube.

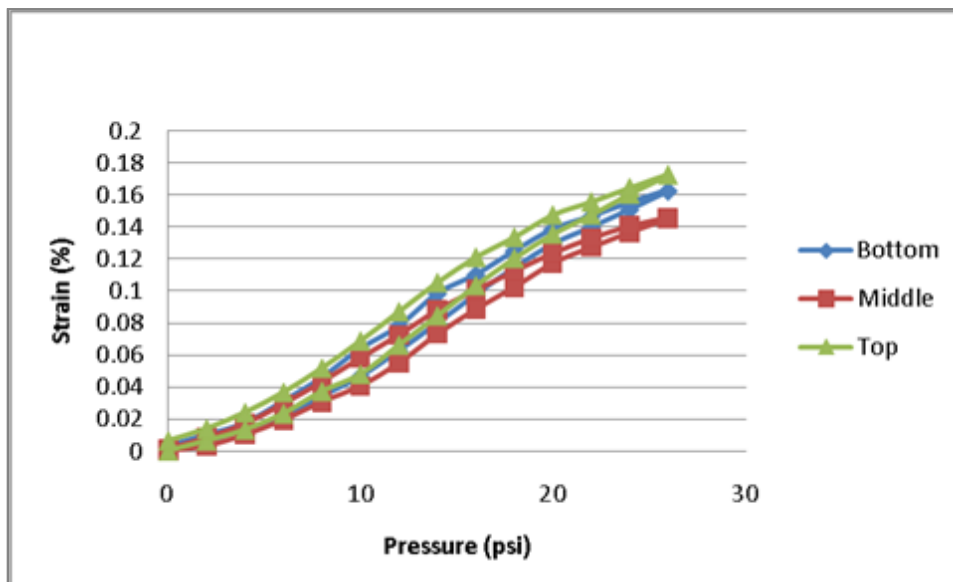


Figure 21 - Strain Values at Varying Tube Locations

The measurements at the bottom of the tube were made on silicon tube that had been cycled at 18psig for five days. These tubes thus seemed to experience some plastic deformation. Shown below is the pressure-outside diameter relationship of the bottom three. The graph shows an initial outer diameter of greater than 1.96mm which is what the silicon tubes are originally. The bottom of the tube shows greater initial deformation

and greater subsequent deformation at increased pressure. The top mark, which is an inch from the bottom of the tube, has only experienced less than two percent plastic deformation (at an initial outer diameter of 1.98mm after 5 days of cycling. After consultation with the client, it was determined that this was not a detrimental issue as the plastic deformation is minimal and the cell rings will not be adversely affected with this change.

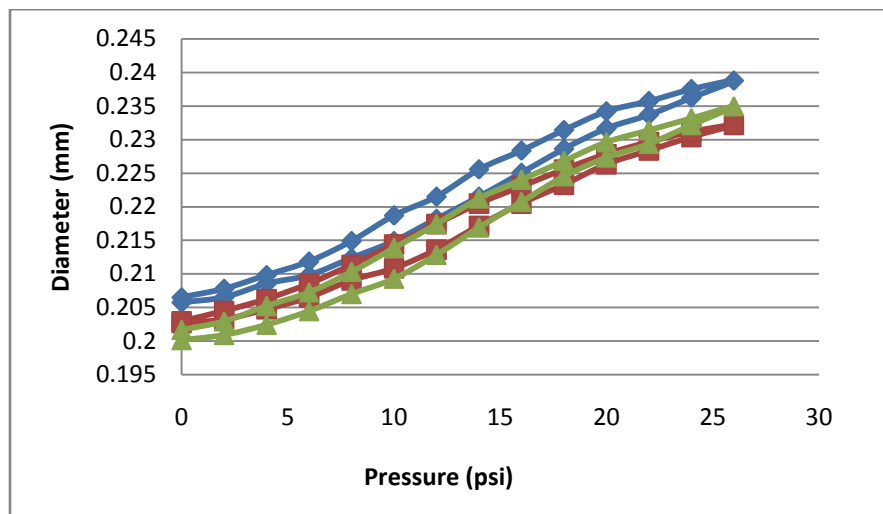


Figure 22 - Outside Diameter Measurements at Varying Tube Locations

Another problem that this experiment revealed was the realignment of the silicone tube during cyclic pressure. The silicone tube would bend to the side and rest against the inside wall of the media chamber, as shown below. To solve this problem, it was determined that an end weight would needed to be added to the bottom of the tube. The final end weight design is shown in the Design Description.

5.2 Pressure Wave Calibration

To induce dynamic strain conditions on the silicon tube, a pressure system would need to be accordingly designed. Needs of the pressure system include the ability to induce the tubes to strain between two distinct diameters, that the frequency of this alternation be approximately 1 Hz, and that the transition between the two effective diameters of the tubes be smooth. These needs were communicated to the team by the client.

A schematic of the basic structure of the pressure system is shown in the figure below. The system begins with a high pressure source, I, of around 120 psig (readily available to the Rolle Lab). This pressure feeds into a control system, II, which outputs a pressure wave into a distributor, III. The distributor, or manifold, distributes that pressure wave into each of eight tube assemblies, IV, which were previously discussed in more detail. The amplitude and period of the pressure wave is assumed to directly correspond to the required strain wave of the tubes.

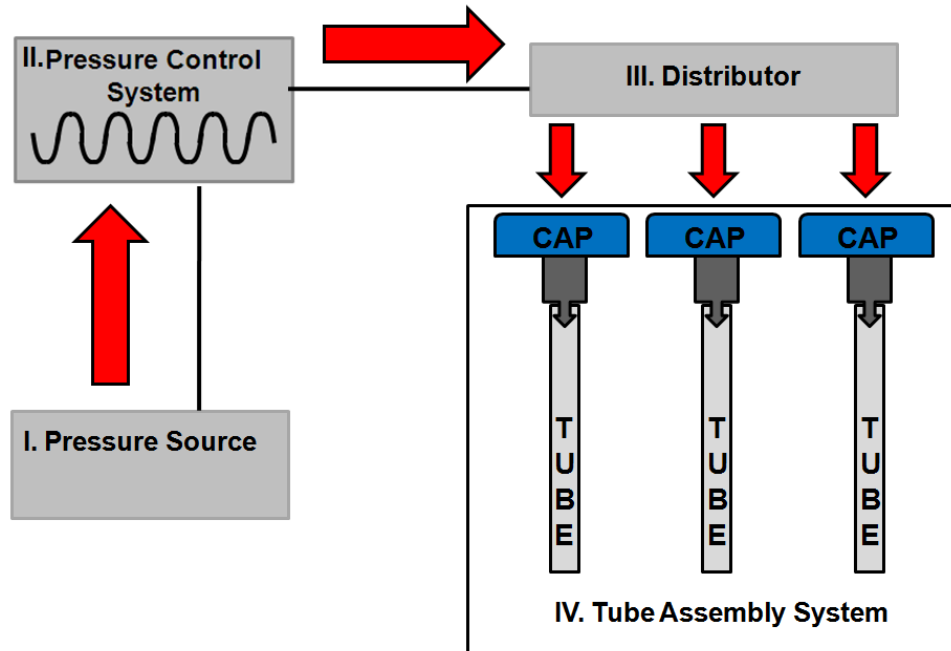


Figure 23 - Dynamic Pressure System

Initially, the team designed a control system that used two similar pressure regulators and a timed, 3-way solenoid valve, shown in Figure 24. The regulators were both supplied equal inlet pressures of ~120psig and set to 4psig and 26psig respectively. The two outlets were individually fed through two “dampening” needle valves and then to two inlets of a 3-way solenoid valve. The output of the solenoid valve was intended to switch between the two different inlet pressures so that the affected silicone tubes would strain between two specific diameters accordingly; the dampening needle valves were to be adjusted so to control the rate of air flow into and out of the tube system to critically dampen the pressure wave.

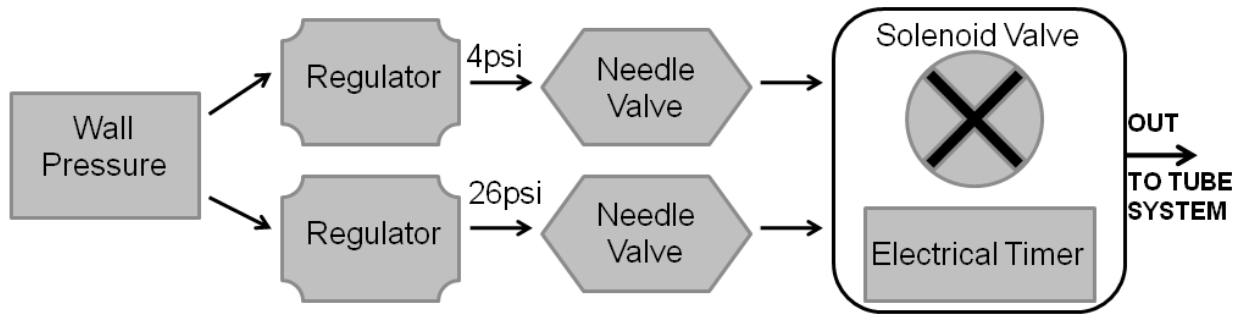


Figure 24 - Original Pressure Control System Schematic

When tested, the high-pressure regulator would quickly and accurately pressurize the tube system, but when the solenoid valve switched from ‘high’ to ‘low’ (inlet pressures), the low-pressure regulator, set to 4psig, would not dissipate the latent pressure of the system in enough time before the timer switched back to ‘high’. To avoid this problem, the low-pressure regulator was dismissed and its needle valve was left in place and tuned so that the latent pressure of the system would dissipate at a controlled rate. The remaining problem with this method was that the fixed low pressure value was now 0psig (atmosphere), so the needle valve needed to be tuned to leak air to the atmosphere slowly enough so that the system’s pressure would descend and reach 4psig at the instant that the solenoid valve switched back to ‘high’. This effectively produced an over-damped wave shape for pressure descent and a critically damped wave shape for the ascent (see Figure 26 below). This is less desirable than a completely critically damped wave because an over-damped wave is not as smooth as one that is critically damped. The experimental procedures that yielded this result are outlined in Appendices E and F.

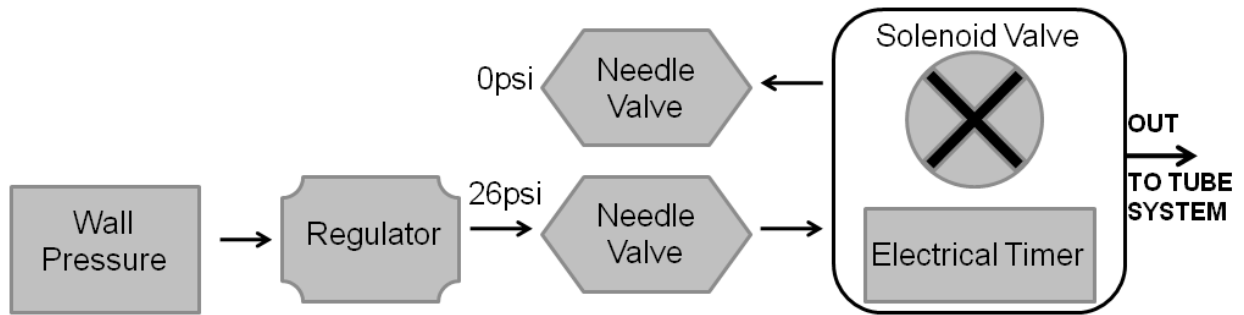


Figure 25 - Single Regulator Pressure Control System Schematic

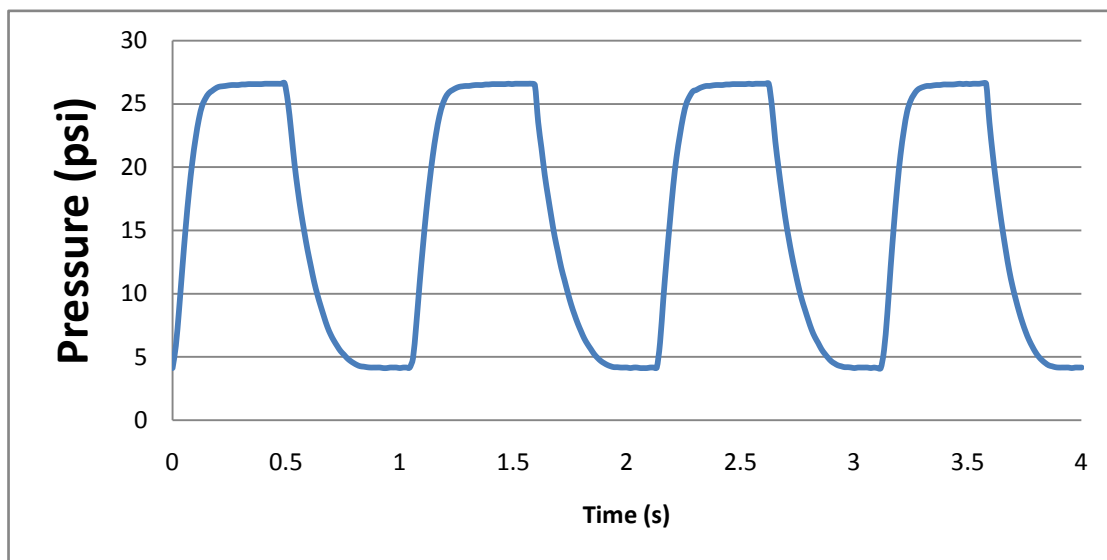


Figure 26 - Single Regulator Pressure Waveform

The task of the verification is thus to determine how to achieve a pressure wave that is entirely critically damped and that cycles between a high pressure of 26psig and a low pressure of 4psig. The team innovated a system, shown next in Figure 27, that introduces an air volume tank to accomplish this wave shape. The 2L air volume tank replaces the low pressure-regulator of the first design. The open end of the tank is fitted with a “control” needle valve. Upon the solenoid valve’s switch from ‘high’ to ‘low’, air from the tube system leaks into the air volume tank (as motivated by the differential

between the latent pressure of the tube system and that of the tank). The tank's internal pressure starts at 0psig, but after a few cycles there is a positive gage pressure within the tank because the tank's end needle valve does not allow the tank to immediately regulate to atmospheric pressure. Instead, the event of the solenoid valve's high-low switch reveals a decrease in the system's pressure whose rate quickly decreases due to the lessening pressure differential between the tank and the tube system. When the needle valves are correctly adjusted, the pressure nearly settles at 4psig.

A pressure-time wave was recorded when the “dampening” needle valves were completely open (low flow resistance) to verify the design's intent to ‘settle’ at a specific low pressure value. Figure 35 in Appendix C shows this wave to be under damped with upper and lower limits of 26psig and 4psig respectively. The slope of the waveform near the bottom of a cycle (~4psig) seems to approach 4psig in a very similar manner that the upper portion of a cycle approaches 26psig. This suggests that the system can be used to achieve near-critical damping. Figure 28 is a pressure-time record of the tube system when the dampening needle valves are correctly adjusted. The figure shows a wave shape quite similar to that of a critically dampened wave.

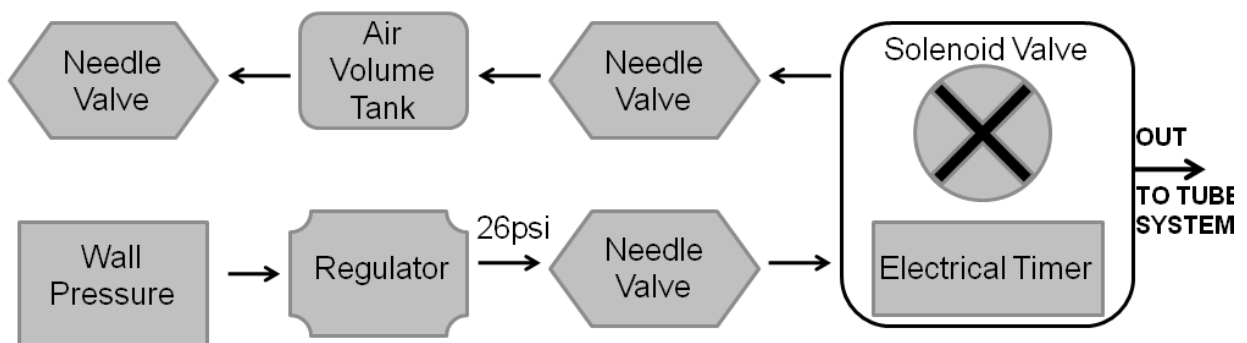


Figure 27 - Air Volume Tank Pressure Control System Schematic

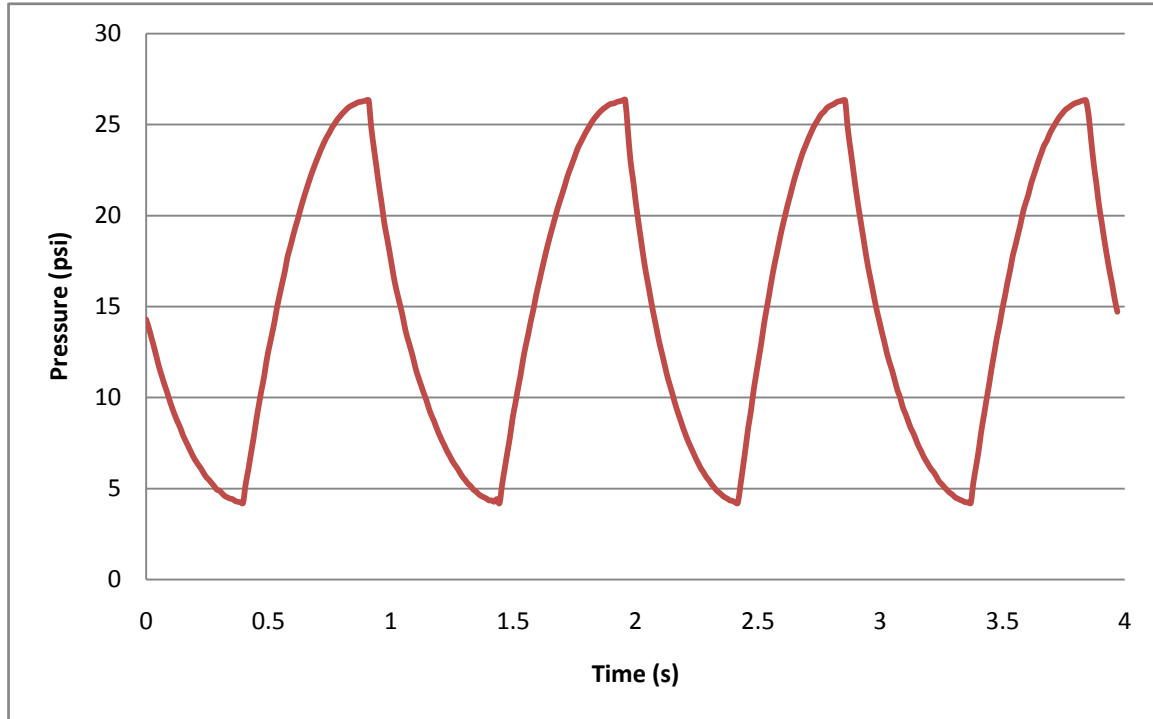


Figure 28 - Critically Damped System Pressure Waveform

5.3 Preliminary Assembly Verification

Another test was conducted to verify the performance of several subsystems at a single time. The nature of the test was to connect several fundamental components of the machine together, and run a mock mechanical conditioning cycle. The components in effect for this test were the timer, the 3-way solenoid valve, the regulators, a needle valve, the manifold, all ball valves, and the cap assembly for eight silicon tube segments.

Setting this test up gave the team much insight into the specific methodology that will be later described to the user. Eight tubes were fitted to eight barb connectors respectively and a heat sleeve was shrunk over each. To seal the ends, small binder clips were used as the end plug method was still in its development stage. One regulator was

set to 2 psig and the other to 20 psig (as monitored by a pressure gage). Electrical energy and air pressure were supplied to the appropriate components of the system and the tubes were allowed to inflate and deflate over a 1 Hz cycle.

Upon initiating the system, several tubes immediately burst near the barb connector. The other tubes' cyclical strain was visibly noticeable. An interesting observation was that one tube seemed to inflate much more at the barb than did the others. A noted difference with this tube was that its heat sleeve covered the majority of the barb, but not the entire thing, whereas the others' heat sleeves did cover the entire barb. This result led to the conjecture that it was not intense heat that causes bursting of the tubes, but rather thinnest-walled section of the tube to inflate like the rest of it. It was thus determined that the heat sleeves need to encase the entire barb.

5.4 Method of Mounting Cell Rings

Another test was executed to verify that the cellular rings intended to be mechanically conditioned were able to be mounted to the silicon tubes by a biologist. To this end, cell rings were grown and a small segment of silicon tubing was obtained. With various laboratory tools and methods, a biologist attempted to fit a ring over the outer diameter of the tube segment. No cell ring was successfully loaded on to a tube. Problems observed were the fact that the rings contracted when they were removed from their culture wells and therefore became much harder to fit over a tube.

A potential way around this problem is the use of a special end plug for the tube. This end plug will have a thin needle or metal rod protruding from its center. This rod would then be used to pierce the center of the post of agarose around which the rings are grown. This would allow the end of the tube to be directly centered on top of the post.

The needle will anchor the agarose wells into place, while the experimenter could then move the cell ring up and on to the tube without any opportunity to contract. A schematic of the needle endcap is shown previously in the Alternate design section. A schematic of the final design is shown in Fig 29, below.

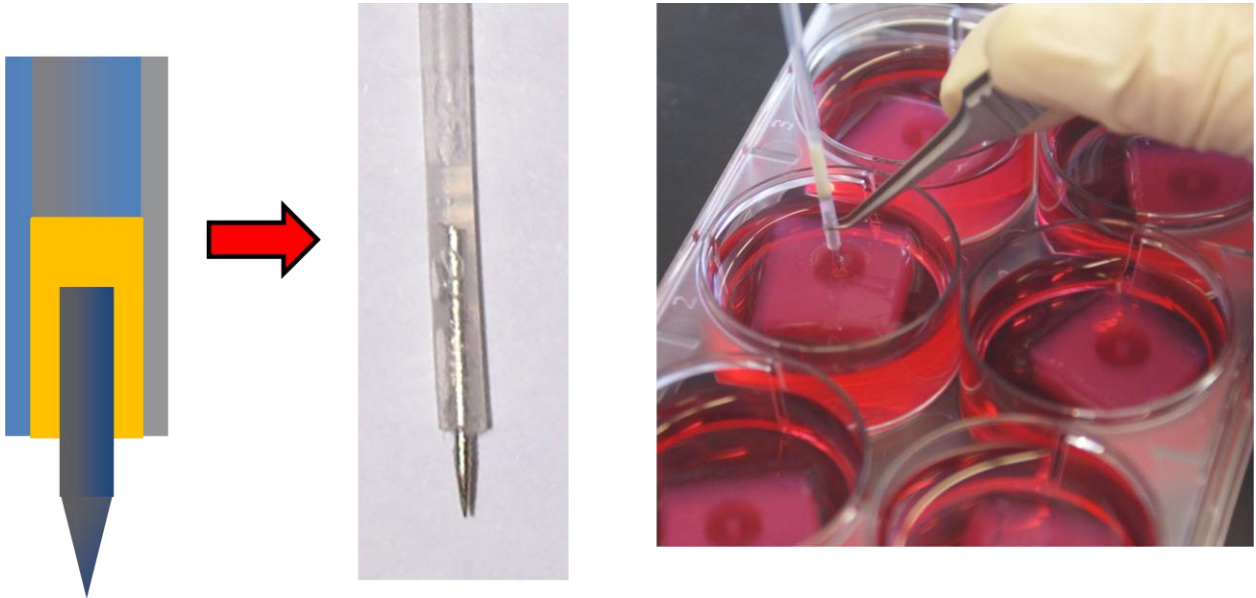


Figure 29 - Needle Endcap Schematic, Actual, and Implementation

5.5 Verification of Media Chambers

The vascular rings and the silicone tube assembly need to stay protected from the outside environment throughout the experiment. To protect the vascular rings, it was determined that media chambers must provide an airtight and secure seal between the internal and external environments. Through testing, it was verified that the media chambers used to store biological media do provide an air tight and safe seal from the outside environment. I was also determined that the media chambers were autoclavable.

6.0 Final Design

After many different design concepts, discussions with the client, verification of system subcomponents and ranking among similar concepts, a final design concept was reached. The design schematic for the final expandable tube system is shown in Figures 30 and 31.

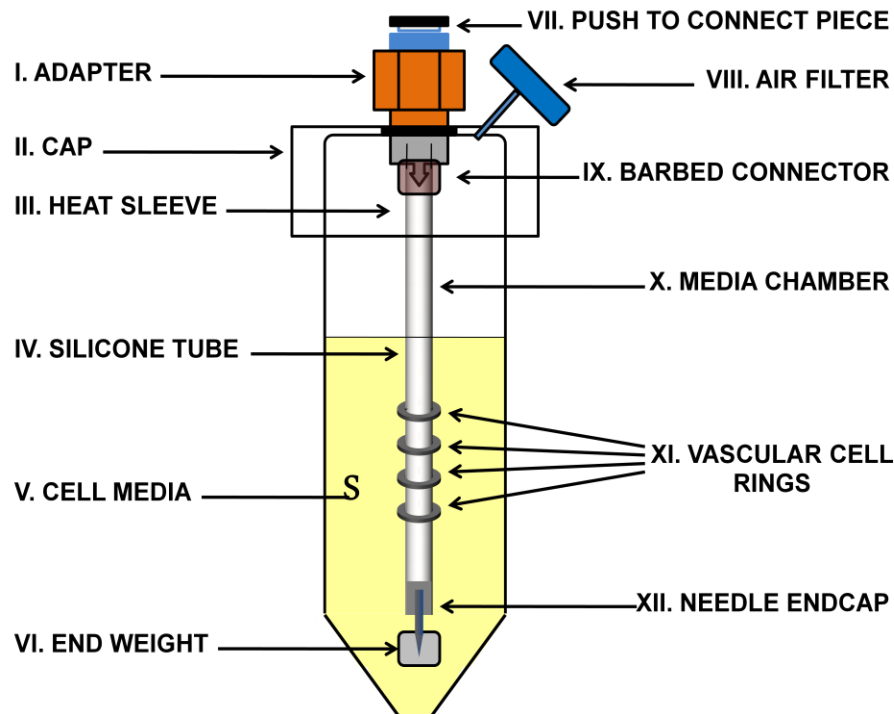


Figure 30 - Final Expandable Tube System Schematic

As seen in the diagram, four tissue-engineered vascular rings, XI, are placed on flexible, inflatable tubing. To ensure non-cytotoxicity and the appropriate mechanical strength for dynamic loading, silicone, IV, is recognized and verified as a suitable option. The outside diameter of the silicone tubing is slightly smaller than the vascular rings. The flexible silicone tubing is connected to a threaded barb, IX, by a heat-sensitive shrinking sleeve, III. The threaded barb screws onto the cap, II, of a media chamber. A hole is drilled through the cap to ensure that the threaded barb fits tightly on the cap. An air

pressure fitting, consisting of an adapter, I, and a push-to-connect piece, VII, screws on to the top of the barb on the opposite side of the cap. On the underside of the media chamber cap, between the cap and the barb connector's hex head, an o-ring, ensures that there is no leaking between the hole made in the cap and the outside environment. The barb and the silicone tube are housed inside the media chamber, X, with the appropriate media, V. A sterile air filter, VIII, attaches to the cap to allow the media to obtain oxygen. The needle endcap design, XII, explained in the Alternate Design section is used to seal the end of the silicone tube. Silicone glue is used as the adhesive between the silicone tube and the needle, as it verified to be strong enough to resist operating pressure and is biocompatible. After the vascular rings are loaded, the needle endcap is inserted into an end weight, VI, which ensures the tube will hang vertically during experimentation. There are eight expandable tubing subsystems in the entire system as seen in the final computer model of the design shown in Figure 31, next.

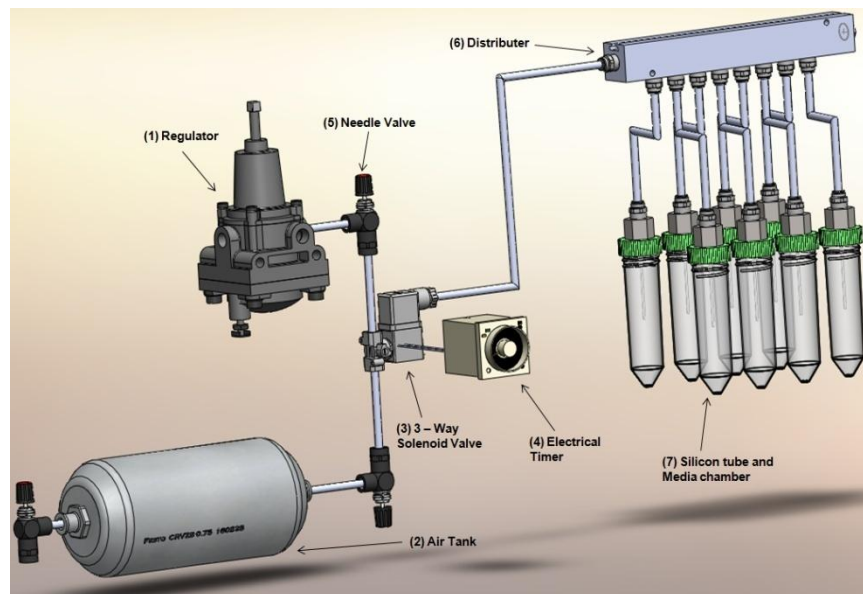


Figure 31 - Final Design Computer Model

A major aspect of the final design is the control of the load that is applied to the tissue-engineered vasculature. In the design, this is done through the use of a pressure control system which includes an air pressure source (from the wall), a pressure regulator (1), an air volume tank (2), a three-way solenoid valve (3), an electrical timer (4), and needle valves (5). The pressure source is connected to the pressure regulator (1), via a standard T-valve. This regulator provides a constant high pressure value of 26psig which is necessary to obtain a 10% strain on the silicone tubing. The output of the regulator is connected to one of the two inputs of the three-way solenoid valve (3). The three-way solenoid valve is controlled by the electrical timer (4) to provide an output that switches back and forth between the high and low pressure inputs. A switch frequency of 0.5 Hz will be set on the timer, effectively controlling the solenoid valve to output the high and low pressures alternately at a frequency of 1Hz. The high-low pressure cycle will thus repeat every second. The air volume tank (2), with assistance of a needle valve, allows the pressure to leak back out of the system, providing a low pressure value. The needle valve on the air volume tank allows the low pressure to be calibrated to only reach the desired level of 4psig instead of emptying the system to 0psig. Two more needle valves (5) are placed between the regulator, air volume tank and the solenoid valve. The needle valves are adjusted and calibrated to ensure critical damping of the system, providing smooth transitions between the high and low pressures.

The output air pressure of the solenoid valve is distributed in parallel to each of the eight aforementioned silicone tubes via a manifold, or distributor (6) and equivalent length, polyurethane tube segments. All other components of the system described above are also connected to each other using polyurethane tube. Directions on how to use the

bioreactor and a full part list can be found in the User Manual in Appendix G. A picture of the final product is shown next in Figure 32.

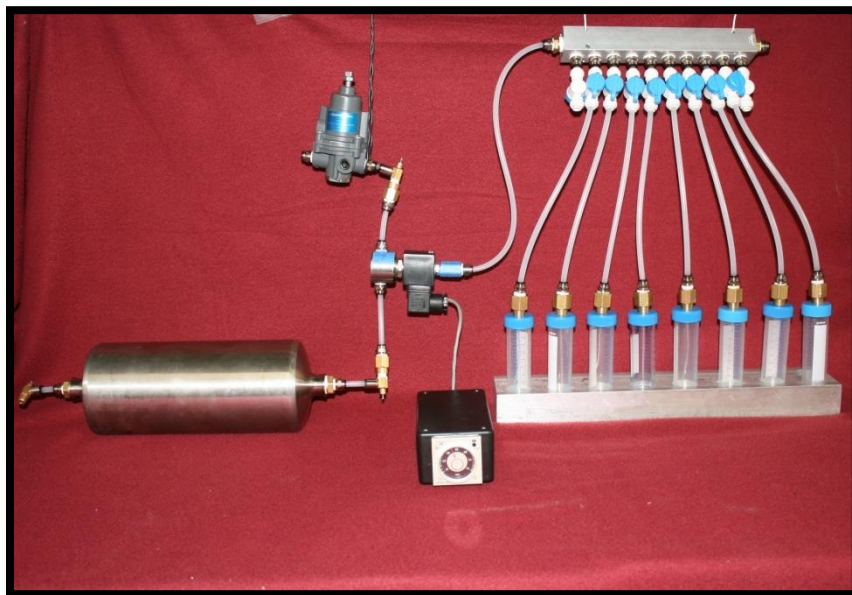


Figure 32 - Final Product

7.0 Conclusions and Recommendations

The team was successfully able to build a mechanical conditioning bioreactor. The bioreactor will be used by the WPI Biomedical Engineering department, specifically the Rolle Lab at Gateway Park, over the summer of 2009 to mechanically condition vascular rings. The bioreactor will cyclically strain the vascular rings at approximately 10% of their original value at a frequency of 1 Hz. The function of system subcomponents and the system as a whole was successfully verified. This included the verification of the pressure control system, the silicone tube, and the silicone tube assembly (barbed connector, heat sleeve and needle endcap).

The team developed a method of controlling the cyclic strain induced on the silicone tube. This method incorporated a pressure regulator, an air volume tank, needle valves and a solenoid valve, which is controlled by an electrical timer. The high pressure is regulated by the pressure regulator and the low pressure is regulated by the air volume tank. A critically damped pressure wave is created by the needle valves which prevents the air in the tubes from being released from the system instantaneously.

An innovative aspect of the teams design is the use of the endcap needle, which makes it easier for an experimenter to load rings onto the silicone tube. The purpose of the endcap needle is to anchor the agarose well in which the vascular cell rings are grown in place so that the experimenter can remove the rings off the posts and onto the silicone tube. This allows a transition from the agarose wells directly onto the silicone tube, as the bottom of the silicone tube aligns with the top of the agarose wells. This method was never validated, due to several challenges. For example, in one attempt, the silicone tube was not cut flush, therefore it did not completely align with the top of the agarose well,

and thus the cell rings were unable to be loaded onto the silicone tube. Another improvement that could be made would be to slightly increase the size of the cell rings. For this purpose, a mold which would make rings of a diameter of 2.25mm, 2.5mm and 2.75mm was made. This will allow the experimenter to optimize the size of the vascular rings by determining which will be the easiest to load onto the silicone tube. Due to time constraints, this was something that was unable to be done by the team due to the long time needed to grow the vascular rings. The process of loading the cell rings onto the silicone needs to be finalized. This was an unexpected problem that the team members faced and thus were unable to plan out from the onset of the project. The timing of the availability of both the rings and the necessary device components did not always work out. Also, rings were lost to contraction issues, breakage, and contamination (Bullock, 2009).

To completely validate the system, the vascular cell rings would need to be cycled on the silicone tube for up to four weeks. The first step in the process would be to cycle a limited number of rings on the silicone tubes, and then try to increase the number of cell rings being cycled up to the desired 4 rings per tube, a total of 32 cell rings. Once the experimenter validates this process, tests can be conducted to determine the effects of varying media conditions on collagen and elastin production. The goal of optimizing media conditions to achieve high strength and appropriate elasticity while being dynamically conditioned can thus be reached.

8.0 References

- Alberts, Bruce, Bray, Dennis, Johnson, Alexander, Lewis, Julian, Raff, Martin, Roberts, Keith, and Walter, Peter. Collagen and Elastin. *Essential Cell Biology, Second Edition*. Garland Science, 2004.
- Berwal and Nouakofski. Collagen and Elastin, 1999.
<http://labs.ansci.uiuc.edu/meatscience/Library/collagen.htm>. Retrieved on November 20, 2008.
- Bullock, Samantha L. Ascorbic Acid Treatment of Fibroblast Tissue Rings to Increase Collagen Production. Worcester Polytechnic Institute, 2009.
- Bunda, Severa, Liu, Peter, Wang, Yanting, Liu Kela, and Hinek, Aleksander. Aldosterone Induces Elastin Production in Cardiac Fibroblasts through Activation of Insulin-Like Growth Factor-I Receptors in a Mineralocorticoid Receptor-Independent Manner. *The American Journal of Pathology*, 2007.
- Ching-Hsin Ku , Philip H. Johnson , Puspa Batten , Padmini Sarathchandra , Rachel C. Chambers , Patricia M. Taylor , Magdi H. Yacoub , and Adrian H. Chester. Collagen synthesis by mesenchymal stem cells and aortic valve interstitial cells in response to mechanical stretch. Cardiovascular Research Advance Access published on August 1, 2006, DOI 10.1016/j.cardiores.2006.03.022. Cardiovasc Res 71: 548-556.
- Cummings, Christopher L., Gawlitta , Debby, Nerem, Robert M., Stegemann, Jan P. Properties of engineered vascular constructs made from collagen, fibrin, and collagen–fibrin mixtures. *Biomaterials*, 2004.
- Currey, John D. Hierarchies in Biomineral Structures. *Science* 309 (5732), 253, 2005.
- Dartsch, P. C., and H. Hammerle. Orientation response of arterial smooth muscle cells to mechanical stimulation. *Eur. J. Cell Biol.*, 1986.
- Davidson, Jeffrey, LuValle, Phyllis, Zoia, Ornella, Quaglino, Daniela, and Giro, MariaGabriella. Ascorbate Differentially Regulates Elastin and Collagen Biosynthesis in Vascular Smooth Muscle Cells and Skin Fibroblasts by Pretranslational Mechanisms. *Journal of Biological Chemistry*, 1997.
- Eagle, Kim A., and Guyton, Robert A. ACC/AHA 2004 Guideline Update for Coronary Artery Bypass Graft Surgery. Journal of the American Heart Association. 2004.
- Freed, L.E. and Vunjak-Novakovic, G. Tissue Engineering Bioreactors. *Principles of Tissue Engineering*, 2000.
- Girton, T. S., V. H. Barocas, and R. T. Tranquillo. Confined compression of a tissue

- equivalent: Collagen fibril and cell alignment in response to anisotropic strain. *J. Biomech. Eng.*, 2002.
- Isenberg, Brett C., and Tranquillo, Robert T. Long-Term Cyclic Distention Enhances the Mechanical Properties of Collagen-Based Media Equivalents. *Annals of Biomedical Engineering* (31) 2003.
- Isenberg, Brett C., Williams, Chrysanthi, and Tranquillo, Robert T. Small-Diameter Artificial Arteries Engineered in Vitro. *Circulation Research*. 2006.
- Iwasaki, K., Kojima, K., Kodama, S., Paz, A.C., Chambers, M., Umezumi, M., Vacanti, C.A. Bioengineered three-layered robust and elastic artery using hemodynamically-equivalent pulsatile bioreactor. *Circulation* 118, 2008.
- Kim, B. S., J. Nikolovski, J. Bonadio, and D. J. Mooney. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nature Biotechnol.*, 1999.
- Kolpakov, V., M. D. Rekhater, D. Gordon, W. H. Wang, and T. J. Kulik. Effect of mechanical forces on growth and matrix protein synthesis in the *in vitro* pulmonary artery – Analysis of the role of individual cell types. *Circ. Res.*, 1995.
- Lee, R. T., C. Yamamoto, Y. Feng, S. Potter-Perigo, W. H. Briggs, K. T. Landschulz, T. G. Turi, J. F. Thompson, P. Libby, and T. N. Wight. Mechanical strain induces specific changes in the synthesis and organization of proteoglycans by vascular smooth muscle cells. *J. Biol. Chem.*, 2001.
- L'Heureux, Nicolas, McAllister, Todd N., and De la Fuente, Luis M. Tissue-Engineered Blood Vessel for Adult Arterial REvascularization. *New England Journal of Medicine*. (357) 2007.
- Liu, S. Q. Influence of tensile strain on smooth muscle cell orientation in rat blood vessels. *J. Biomech. Eng.*, 1998.
- Michaels, Andrew D., Chatterjee, Kanu. Angioplasty Versus Bypass Surgery for Coronary Artery Disease. *Circulation*. (106) 2002.
- O'Callaghan, C. J., and B. Williams. Mechanical strain-induced extracellular matrix production by human vascular smooth muscle cells: Role of $\text{tgf-}\beta(1)$. *Hypertension*, 2000.
- Parks, Robin. "Coronary artery bypass graft (CABG) surgery." 14 May 2007. WebMD. <www.webmd.com>.
- Parmer, Sharon. Coronary Artery Bypass Grafting. *JAMA*. (299) 2008.

- Sahoo, Sambit, Cho-Hong, James G., Siew-Lok, Toh. Development of hybrid scaffolds for potential applications in ligament and tendon tissue engineering. *Biomedical Materials*, 2007.
- Shastri, P. Nano and Micro-Scale Engineering of Synthetic Vascular Grafts, 2004.
- Seliktar, D., R. A. Black, R. P. Vito, and R. M. Nerem. Dynamic mechanical conditioning of collagen-gel blood vessel constructs induces remodeling *in vitro*. *Ann. Biomed. Eng.*, 2000.
- Schaffer, Amanda. Better Blood Vessels. Technology Review Published by MIT. 2007.
- Sung In Jeong, Jae Hyun Kwon, Jin Ik Lim, Seung-Woo Cho, Youngmee Jung, Won Jun Sung, Soo Hyun Kim, Young Ha Kim, Young Moo Lee, Byung-Soo Kim, Cha Yong Choi, Soo-Ja Kim. Mechano-active tissue engineering of vascular smooth muscle using pulsatile perfusion bioreactors and elastic PLCL scaffolds. *Biomaterials*, Volume 26, Issue 12, April 2005, Pages 1405-1411
- Syedain, Zeeshan, H., Weinberg, Justin S., and Tranquillo, Robert T. Cyclic distention of fibrin-based tissue constructs: Evidence of adaptation during growth of engineered connective tissue. *PNAS* (105) 2008.
- The American Heart Association. 2008. Available at <http://www.americanheart.org>. Accessed September 15, 2008.
- Van der Lei, B., Wildevuur, C.R.H., and Nieuwenhuis, P. Compliance and biodegradation of vascular grafts stimulate the regeneration of elastic laminae in neoarterial tissue: an experimental study in rats. *Surgery*, 1986.
- Vesely, I. The role of elastin in aortic valve mechanics. *J Biomech* 31:115-23, 1998.
- Vunjak-Novakovic, G., Altman, G., Horan, R., and Kaplan, D. Tissue Engineering of Ligaments. *Annual Review of Biomedical Engineering*, 2004.
- Wilson, E., Q. Mai, K. Sudhir, R. H. Weiss, and H. E. Ives. Mechanical strain induces growth of vascular smooth muscle cells via autocrine action of PDGF. *J. Cell Biol.*, 1993.

9.0 Appendix

Appendix A: Specifications for Vessel Ring Bioreactor

1. Pressure Source
 - a. Fluid type needs to be easily accessible and reliable.
 - b. Pressure needs to be generally constant/stable.
2. Rigid tubing
 - a. Needs to maintain rigidity over the pressure and time.
 - b. Cannot corrode or wear.
 - c. Standard size should allow for easy replacement.
 - d. Minimal chance of leakage (e.g. low number of connectors and adaptors).
 - e. Capacitance of the tubing must be controlled.
3. Regulators
 - a. Regulators need to be compatible with the inlet pressure as provided by the pressure source.
 - b. Regulators need to accurately regulate the output pressure over the desired range.
 - c. Regulators need to operate consistently for 4 weeks.
4. Elastic Tubing
 - a. At rest outer diameter needs to be slightly smaller than 2mm, 4mm and 6mm.
 - b. Needs to inflate to 110% of its original diameter within 20psig.
 - c. Surface must allow for the proper adhesion for polymerization of polymer.
 - d. Must not be cytotoxic.
5. Medium Containers
 - a. Must be readily available in large quantities for ease of replacement.
 - b. Must be autoclaveable if not already sterile.
 - c. Must be able to be easily removed and attached to the device.
 - d. Must be able to hold enough medium to support cell life.
6. 3-way Solenoid Valve
 - a. Must be able to switch its outlet pressure between a high pressure at one inlet port and a low pressure at the other inlet port.
 - b. Must be able to operate at 1Hz frequency consistently for a duration of at least 4 weeks.
 - c. Must be operable within the range of its inlet pressures.
7. Valve Controller
 - a. Must be able to switch the solenoid valve between its two states at 1Hz continuously for at least 4 weeks.
 - b. The required voltage to drive it must be safe and available in the provided laboratory space.
 - c. Must be able to turn on and off easily.
8. Tubing Connectors (at all interfaces)
 - a. Must maintain an airtight seal between the two components it connects.

- b. Must be capable of keeping the tubes it connects in place for up to 4 weeks.
 - c. Must allow the passage of pressurized air between the components it joins without significant resistance.
 - d. Must be biocompatible and autoclaveable (applying to the connectors within the culture medium)
- 9. Elastic tube plugs
 - a. Must plug the ends of the elastic tubes without leaking air.
 - b. Must be able to keep its seal within the applied pressure range.
 - c. Must maintain its seal for at least 4 weeks in its pressurized environment.
 - d. Must be biocompatible.
 - e. Must be autoclaveable.
- 10. Medium Container Caps
 - a. Must be threaded so as to allow the medium containers to screw into the caps.
 - b. Must maintain an air-tight seal with the medium containers for at least 4 weeks.
 - c. Must be large enough and thick enough to reliably hold the air filter and the connector between the semi-rigid tubing and the elastic tubing.
 - d. Must be able to be milled using the cutaway milling machines located in Washburn Labs at Worcester Polytechnic Institute.
 - e. Must be biocompatible.
 - f. Must be autoclaveable.
- 11. Air Filters
 - a. Must provide a low-resistance pathway for air displacement in and out of the containers.
 - b. Must prevent all common, harmful material from entering the medium containers.
- 12. Distributor
 - a. Must allow even pressure distribution to eight output ports from a single, pressurized inlet port
 - b. Must be compatible with all connectors and tubes of which it interfaces.
 - c. Must have airtight seals with all connected tubes.

*Note:

- The total effective air capacitance of all subsystems combined must not adversely affect the ultimate pressure characteristic within each elastic tube.
- The total effective air resistance of all subsystems combined must not adversely affect the ultimate pressure characteristic within each elastic tube.

Appendix B: Objective Tree and Pairwise Comparisons

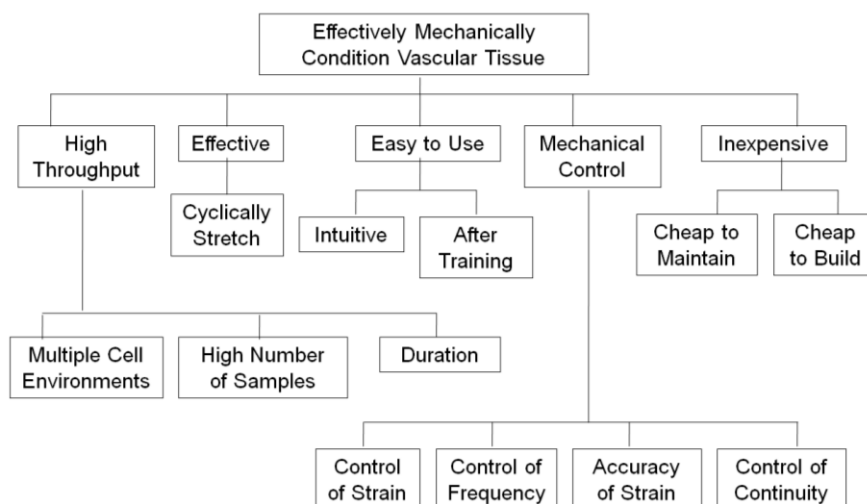


Figure 33 - Objective Tree

Table 3 - Main Objectives Pairwise Comparison

Main Objectives						
	High Throughput	Effective	Mechanical Control	Inexpensive	Easy to Use	TOTAL
High Throughput	X	1	1	1	1	4
Effective	0	X	1	1	1	3
Mechanical Control	0	0	X	1	0	1
Inexpensive	0	0	0	X	0	0
Easy to Use	0	0	1	1	X	2

Table 4 - Mechanical Control Pairwise Comparison

Mechanical Control					
	Continuity	Strain	Frequency	Accuracy of Strain	TOTAL
Continuity	X	0	1	0	1
Strain	1	X	1	1	3
Frequency	0	0	X	0	0
Accuracy of Strain	1	0	1	X	2

Table 5 - High Throughput Pairwise Comparison

High Throughput				
	# Samples	Duration Longevity	Multiple Conditions/Environments	TOTAL
# Samples	X	1	1	2
Duration Longevity	0	X	0	0
Multiple Conditions/Environments	0	1	X	1

Appendix C: Waveforms from Pressure Calibration

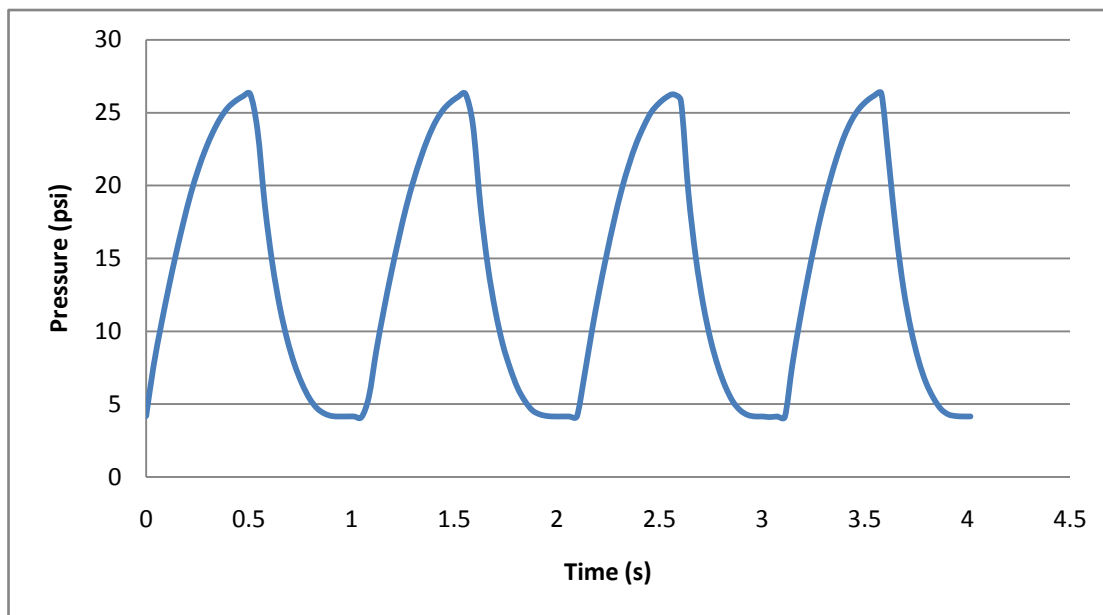


Figure 34 - System Critically Dampened at the High and Under-dampened at the Low

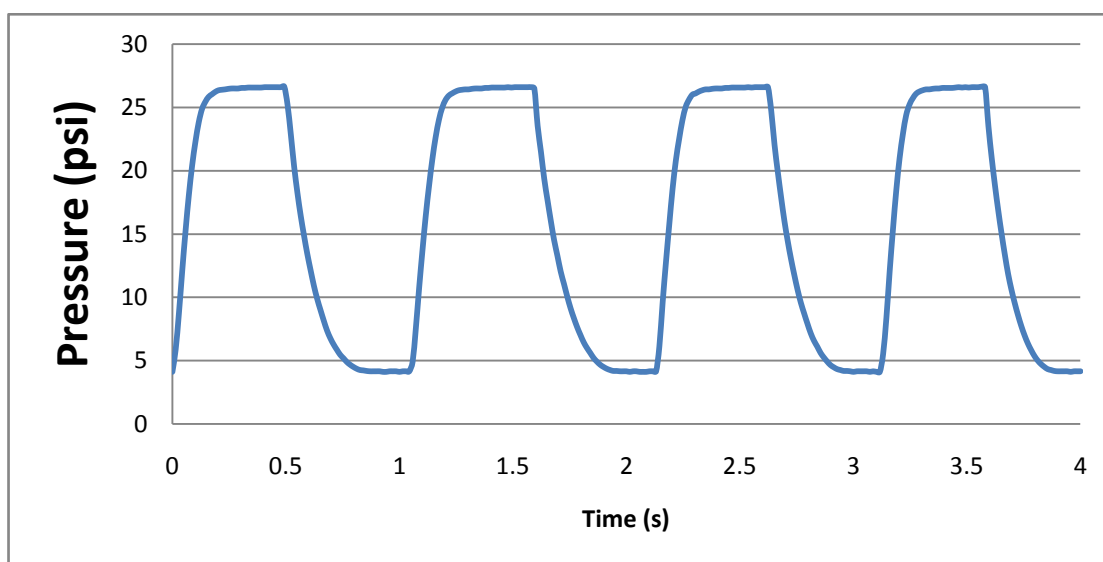


Figure 35 - Completely Under-dampened System

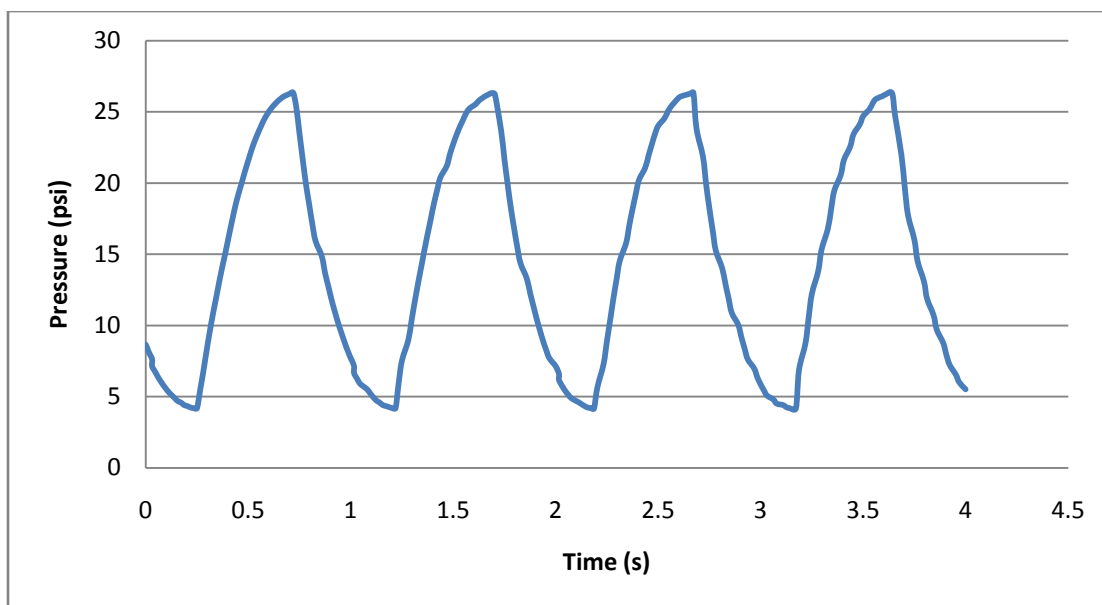


Figure 36 - System Critically Dampened High and Over-dampened Low

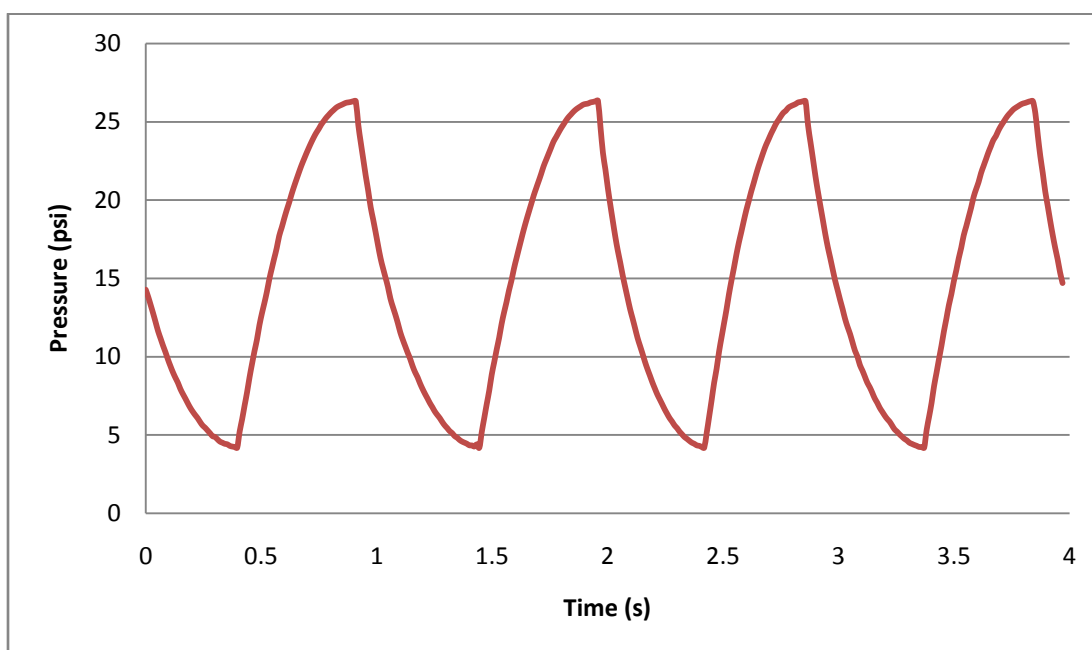


Figure 37 - Critically Dampened System

Appendix D: Protocol for Vertically Testing Silicon Tubing

Purpose: To determine the strain in the silicone tube as a function of pressure

1. Load the cap-silicon tube assembly to vertical fixture. Before this is done, place the heat-sleeve on the silicon tube and heat to get a permanent fit. Place a weight on the end of the tube (binder clip used as both a weight and a seal).
2. Change the orientation of the camera so as to horizontally capture image.
3. Place DVT lighting device adjacent to the DVT camera on the opposite side of the silicon tubing.
4. Run DVT program. Calibrate system by identifying known wire diameter.
5. Identify edges of the silicon tubing on the DVT program.
6. Identify the measurable quantity of “Thickness”.
7. Connect with DataLink to record data.
8. Connect pressure transducer to a ‘T’ connector placed in series between the pressure regulator’s output and the barb connection point.
9. Connect the digital display to the transducer and verify the presence of a single static pressure.
10. Connect the quarter-inch tubing from the barb configuration to pressure source.
11. Label four points along the silicon tube which at 0.5 inch increments from the top of the tube.
12. Make sure that the silicone tube is completely stationary.
13. Inflate tube: Increase pressure to ~26psig by 2psig increments and collect ‘thickness’ at a marked elevation.
14. Repeat process at different elevations (range = 0 – 26 psig).

Appendix E: Protocol for Sub-Assembly Verification

Purpose: To determine the effects of mechanically conditioning one silicone tube within a media chamber filled with water and verify function of all sub-assembly components.

1. Attach elastic tubing to compressor.
2. Use T-valve to attach to inlet of 2 regulators.
3. Using another T-valve, link the output of the two regulators.
4. Using elastic tubing, attach regulator output to inlets of solenoid valve.
5. Using T-valve, bifurcate output of solenoid valve, attaching one end to a pressure transducer and other end to the top of the cylinder cap assembly.
6. Attach tube to barb. Attach tube block on open end and heat-sleeve both ends.
7. Fill cylinder with water and screw onto the cap.
8. Connect electrical wiring to solenoid valve. Connect to voltage input and to time relay, in parallel. This will allow for one voltage source provided to the solenoid valve and the time relay. Set voltage to 12V.
9. Set timer to run at 1 Hz.
10. Set regulated pressure to 1psig and 20psig.
11. Let the system run for 5 days.

Appendix F: Protocol for Verification of Regulators, Timer and Solenoid Valve

Purpose: To verify function of sub-assembly consisting of Solenoid valve, electrical timer and pressure regulator.

1. Electrical timer is connected to voltage source and solenoid valve.
2. Set voltage source to 12 volts.
3. The outlet pressure (from the wall) was connected to a push to connect fitting.
The pressure valve is kept closed until validation.
4. Polyurethane (PUT) tubing was connected to the push to connect fitting on the pressure outlet source. This piece of tubing was connected on the other end to a 3-way fitting.
5. The two remaining outlets on the 3-way fitting were further connected to 2 pieces of PUT tubing.
6. These two tubes were connected to push to connects on the inlet of the two pressure regulators.
7. A push to connect was attached to the outlet of the pressure regulators, which were connected to the respective inlets of the solenoid valve.
8. A pressure gauge was attached to the output of the solenoid valve to measure the outlet pressure.
9. Pressure was varied in the two regulators by adjusting the top screw.
10. Change was visually observed after activation of the solenoid valve by the voltage source.

Appendix G: User Manual and Part List

Forward

The vessel ring bioreactor was built as a Major Qualifying Project at Worcester Polytechnic Institute during the academic school year of 2008-2009 for the Rolle Lab in Gateway Park. This machine was designed to cyclically strain the vessel rings cultured in the Rolle Lab for the purpose of experimentation.

The main design focus was to enable the user to test the effects of cyclic, radial strain on vascular cell rings. The performance of the machine was not validated before this manual was created, but most of the machine's subsystems were verified individually and in combination with each other. Please refer to the MQP report, "Vessel Ring Bioreactor," for verification information.

This user manual was written to provide the user with information on the machine's setup, its calibration and operation. It does not detail how to make vascular cell rings nor does it explain the proper sterile technique required to avoid contamination. It is assumed that the user of this machine is trained in sterile technique and general lab practice. A numbered list of parts as well as ordering information is included at the end of this manual.

Contents

Assembly

- To connect high pressure regulator

- To connect air volume tank

- To connect 3-way solenoid valve

- To connect manifold

- Base and manifold connection

- Tube Assembly

- Filter tube assembly

- End weight construction

Preparation

- High pressure regulator calibration

 - Using a mechanical pressure gage

 - Using a pressure transducer

- Low pressure and waveform calibration

 - Using a mechanical pressure gage

 - Using a pressure transducer

- Cleaning

- Autoclaving

Running the Bioreactor

- Ring loading

- Sterile tube assembly and startup

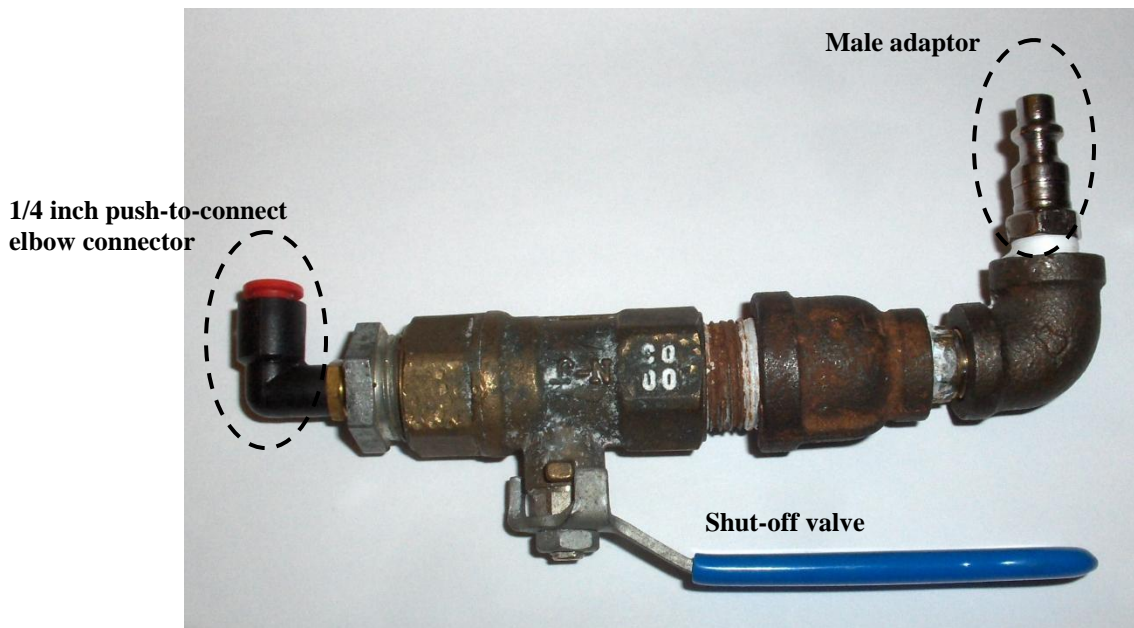
- Changing the media

Assembly

To connect the high pressure regulator:

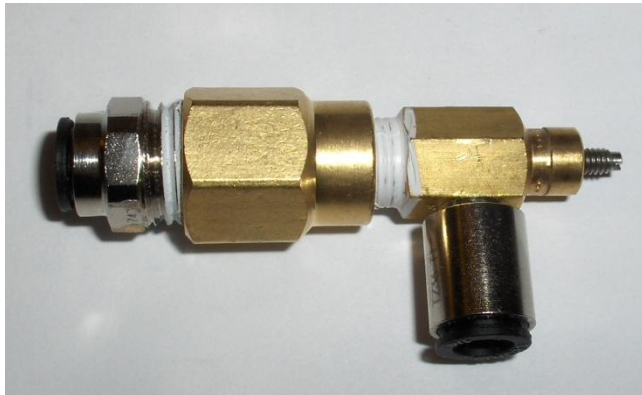
* Note: All air pipe fittings such as the push-to-connect connectors, plugs, adaptors, etc. tighten appropriately without requiring the part to be screwed in completely. All threaded male ports must be wrapped with Teflon pipe tape unless a tape or sealant is already on the part.

The pressure system relies on a continuous, positive pressure source of ~100 psig. The 'house air' in the Rolle Lab provides this. An adaptor is required to properly convert the wall's female fitting to a 1/4 inch push-to-connect fitting. It is recommended to include a shut-off valve at the point of connection (the wall's fitting might already have such a valve in place). An example of an adaptor setup is seen below.



1. Turn the shut-off valve to its 'off' position (perpendicular to pipe's length).
2. Retract the collar on the wall's port and insert the barb connector. Release the collar and give the adaptor a slight tug to ensure it is properly connected.

3. Cut a desired length of 1/4 inch LDPE tubing using a razor blade. Ensure that the cut is nearly perpendicular to the tube's length. Insert one end into the push-to-connect port. Give it a slight tug to ensure it is properly inserted.
4. Insert a 1/4 inch NPT push-to-connect connector (part #9) into the "IN" and "OUT" ports (as seen on the underside) of the air pressure regulator. You may use the 14mm wrench.
5. Insert the remaining end of the LDPE tube that is now connected to the wall's port into the "IN" port.
6. Using a 5/16 wrench, turn the adjusting screw of the regulator counter-clockwise until it becomes completely loose. Then, turn it clockwise 1 full turn so it does not fall out. This will prevent any airflow out of the regulator.
7. Cut another LDPE tube of desired length and insert one end into the push-to-connect connector located in the "OUT" port.
8. Assemble a needle valve (part #8) with a 1/8 – 1/4 inch female-female bushing (part #12) and a 1/4 inch push-to-connect connector. Insert the appropriate 1/4 push-to-connect connector in the remaining port as shown below.



9. Insert the remaining end of the LDPE tube into any port of the needle valve.

To connect the air volume tank:

1. Insert a 1/2-1/4 male-female bushing into both ends of the air volume tank.
2. Insert a 1/4 inch push-to-connect connector into both bushings.
3. Cut two small sections of LDPE tubing and insert one into both ends of the tank.
4. Assemble another needle valve with one push-to connect connector and mount it on the other side of one of the small tubes.
5. Assemble another needle valve with two push-to-connect connectors and insert the remaining small tube into one of its ports as seen below.

**To connect the 3-way solenoid valve:**

*Note: The two inlet ports are NOT #1 and #2 although it may look like it should be.

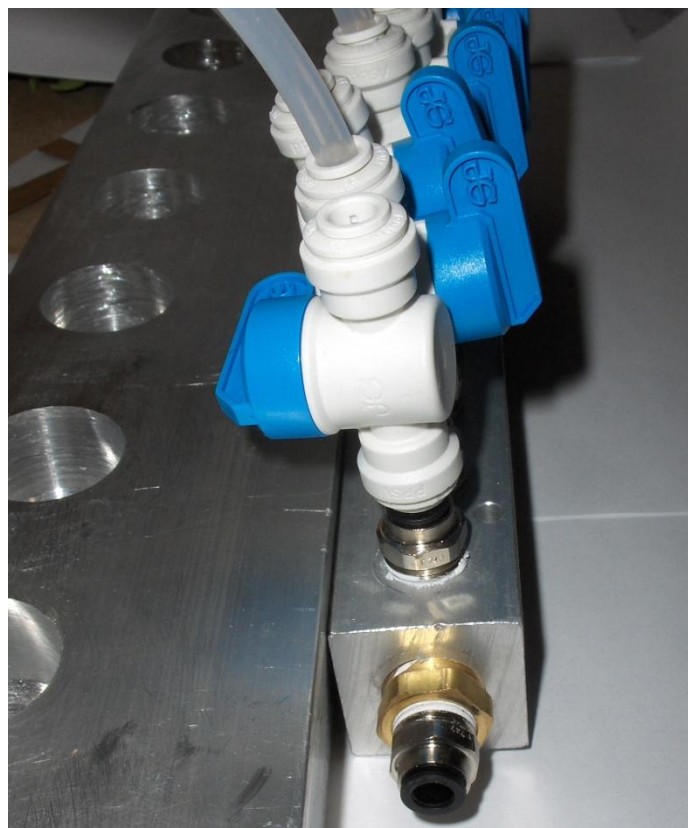
1. Insert a 1/4 inch push-to-connect connector into each of the three ports.
2. Cut a desired length of LPDE tubing and connect port #2 to the remaining port of the high pressure regulator's needle valve.
3. Cut a desired length of LPDE tubing and connect port #3 (it is not labeled, but it is the remaining port; i.e. not #1 or #2) to the remaining port of the air volume tank's needle valve with two ports.
4. Port #1 is the output of the solenoid valve. Connect a desired length of LDPE tubing to this port.

To connect the manifold:

1. Insert a 3/8-1/4 male-female bushing into both ends of the manifold.
2. Insert a 1/4 inch push-to-connect connector into each of nine outlet ports (all on the same side of the manifold).
3. Insert a 1/4 inch push-to-connect connector into one bushing an end of the manifold.
4. Insert a 1/4 inch NPT brass plug (part #27) into the other end of the manifold and another plug into the remaining open outlet on the main side of the manifold.
5. Cut nine 1.5 inch lengths of LDPE tubing and connect them to the nine ports on the main side of the manifold.
6. Connect eight, ball valves (part #7) to eight of the short tubes on the main side of the manifold.
7. Turn 'off' all ball valves so that the handles are perpendicular to the air flow.
8. Connect a mechanical pressure gage (or electrical transducer) to the remaining short LDPE tube.
9. To each of the ball valves, attach a fixed length of LDPE tubing (around 20 inches long). You may cut these tubes into sections and use the elbow connectors (part #19) to arrange them to desired heights (see Base and Manifold connection).
10. Connect eight more ball valves to the remaining ends of the LDPE tubes of fixed length from the previous step.
11. Connect the remaining end of the LDPE tube exiting the output port of the 3-way solenoid valve to the remaining push-to-connect connector on the end of the manifold.
12. Cut eight more LDPE tubes at 4 inches long. Wipe with alcohol or other cleaning agent and set aside in a clean environment. These will be used later during preparation.

Base and manifold connection:

1. Position one side of the manifold to the side of the base furthest from the base's holes as shown in the two pictures below.



2. Align the manifold's two screw holes with the tapped holes in the base and insert screws. Ensure that the screws are snug but not too tight.

Tube assembly:

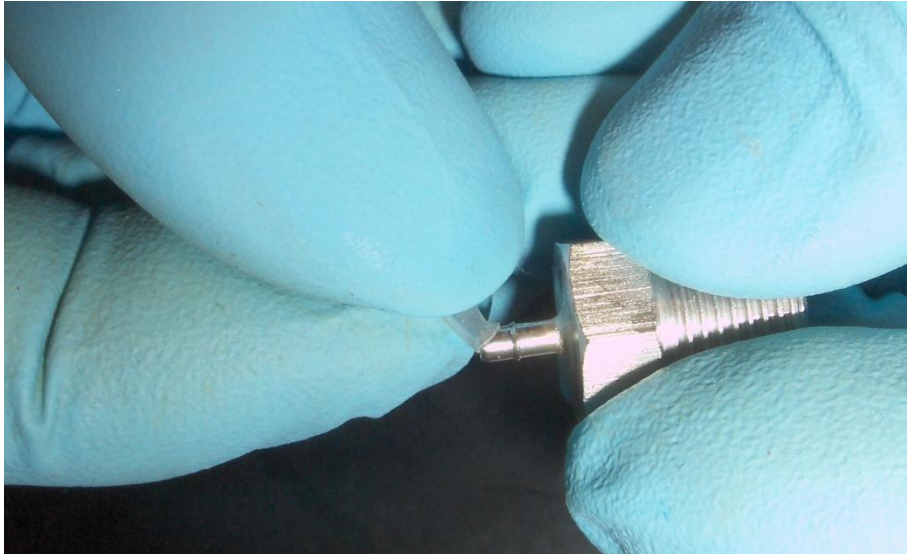
*Note: It is recommended that the user handle the silicone tubes while wearing appropriate gloves to reduce the presence of oil and dirt.

1. From the 50 ft length of silicone tubing, cut eight tubes to 3.5 inches long. Be sure that the cut is exactly perpendicular to its length. This will affect the ability to load cell rings onto the tubes.
2. Using wire cutters, cut a ~0.25 inch section of the needle off of eight syringes (part #22). Ensure that the sharp end is included in this cut section.
3. Obtain medical grade silicone glue (found in the lab) and fill one end of each silicone tube. The glue should have no visible bubbles or air pockets and should also extend up the tube about 3/8 inch. A promising technique is to first apply a small amount of glue to your index finger, and then scrape it off with the edge of the silicone tube. Repeating this process pushes more and more glue into the tube. This will take practice.
4. Wipe away any residual glue that may have gotten on the outside of the tube.
5. Insert the cut needle section into the center of the still-wet glue so that the sharp end remains pointing out of the tube. Ensure that only a small portion of the needle is exposed (~1/8 inch). You must also ensure that the needle is as close as possible to coaxial alignment with the tube. The needle must NOT poke through the glue (i.e. leave enough glue behind the needle to ensure the end is sealed once the glue cures).
6. Repeat step 4 for all remaining tubes and allow the glue to completely cure. The end of the tube should look like the picture below.

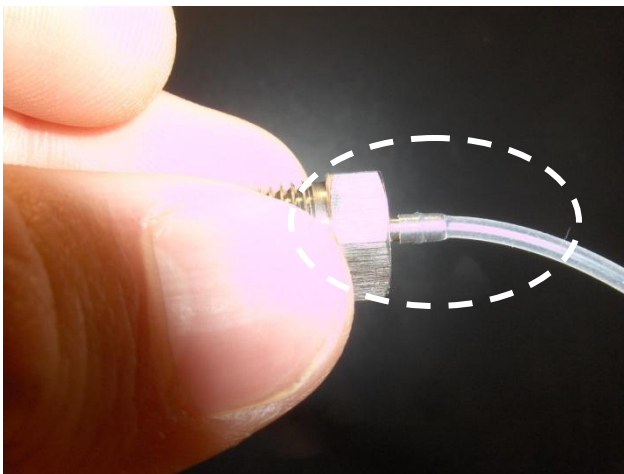


7. After the glue is cured, locate eight of the barbed tube connectors (part #15).

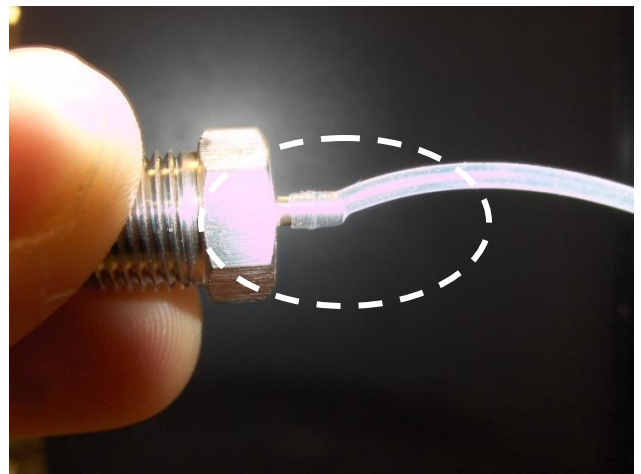
8. On each, fix the open end of a silicone tube around the barb. Accomplishing this without overly straining the tube's end can be a difficult task. Try pushing the end of the tube onto the barb starting at a 45° angle and rotating it while gradually pushing it on the rest of the way (refer to picture below). Be sure that the tube aligns well with the barb as seen in the 'correct' picture.



Initially pushing tube at 45° angle

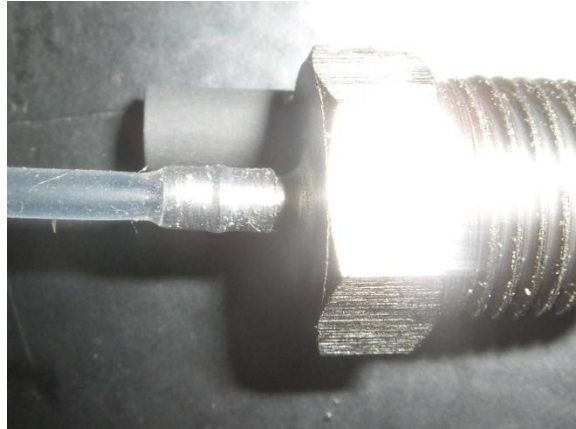


Correct Alignment



Incorrect Alignment

9. With a razor blade, cut a 1/4 inch length of the heat sleeve tube (part #31) for each of the eight barbs. With your eyesight, ensure that the cut is almost exactly perpendicular to the tube's length. The length of the heat sleeve should compare to the length of the barb as shown below (i.e. *slightly* longer than the barb itself).

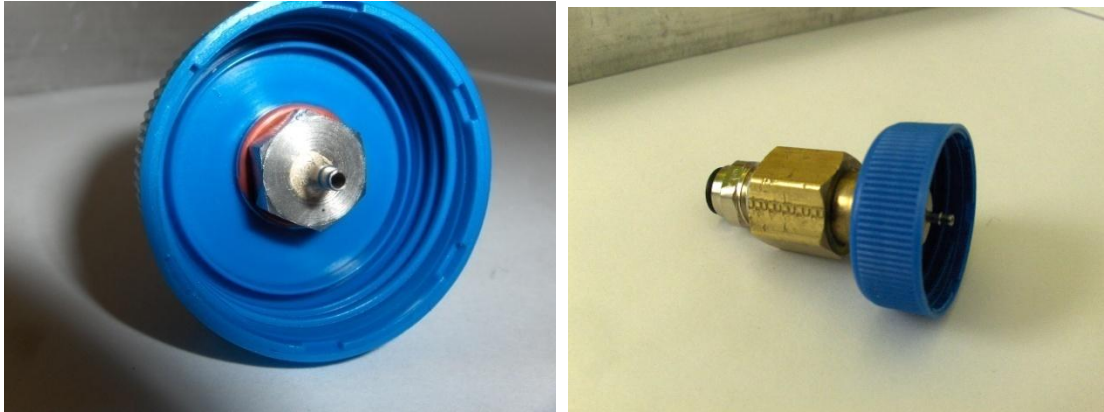


Sleeve is slightly longer than barb

10. Position the sleeve around the silicone tube and slide it until it butts up against the hex head face of the barb connector.
11. Turn on a heat gun (supplied in the lab) and set its temperature to 315° C.
12. Apply uniform heat to the entire outer surface of the heat sleeve. Do this by holding the silicone tube near the needle end so the barb hangs vertically (refer to picture below). Using your index finger and thumb, slowly roll the tube so that it rotates gently. While rotating the tube, aim the heat gun at the sleeve at a downward angle while keeping the gun's tip about an inch away from the tube. The entire process for the sleeve to shrink around the silicone tube should take less than two seconds.



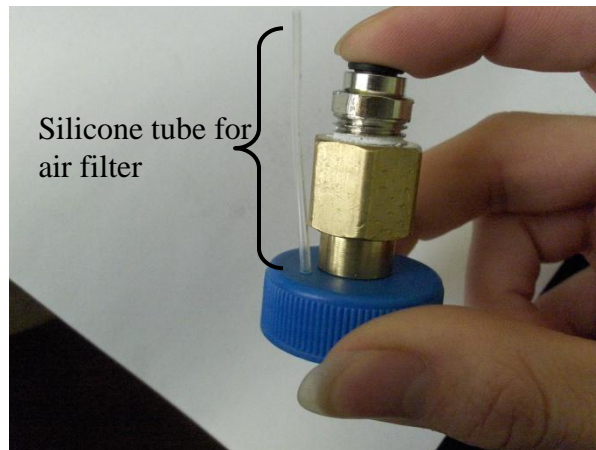
13. Apply Teflon plumbing tape to the threads of each. The direction of wrapping the tape should be clockwise if you are holding the hex head with the threads facing you.
14. Fit an FEP encapsulated silicone o-ring (part #21) over the threaded section of each barb. Push it all the way against the hex head.
15. Insert the threaded portion of each barb through the center hole in each media container cap (hole is drilled with a 13/32 inch drill bit).
16. Screw the appropriate end of a 1/8-1/4 inch female-female bushing onto the barb until it presses snugly against the face of the cap. You may use the 11mm wrench for the barb connector and the 3/4 inch wrench for the bushing. **BE CAREFUL TO NOT BEND THE BARB.** While tightening the two parts, a wrench may slide off the hex head and strike the barb causing it to bend. A good way to test the tightness of fit is to attempt rotating the cap. If you can rotate it using slightly less torque than you generally use to turn a smooth doorknob, it is tight enough.
17. Insert and tighten a 1/4 push-to-connect connector in the open end of each bushing. A picture of the setup is next.



Filter tube assembly:

*Note: Prior to the writing of this manual, an air filter was never applied. The following instructions explain intended methods, not tested methods.

1. Cut a 2 inch length of silicone tube and insert it into off-centered hole in each cap [hole size for the silicone tubing used on the barbs is 0.07 inch (use -012 drill bit)]. See picture.



2. Apply silicone glue around the outside of the tube at the junction of the tube and the cap.
3. Repeat step 2 for all caps and allow for glue to cure.

The purpose of gluing this tube onto the cap is to insert the end of an air filter into

the tube. The air filter should not be applied at this stage. It will be applied after the cap/tube assembly is autoclaved.

End weight construction:

*Note: Prior to the writing of this manual, an end weight was never applied. The following instructions explain intended methods, not tested methods.

1. Locate eight stainless steel acorn nuts (part #30).
2. Fill the inside (where the threads are) of each nut with PMMA or other silicone elastomer.
3. Allow all end weights to cure.

Eventually, the needle endplug will insert into the cured PMMA of the end weight.

Preparation

High pressure regulator calibration:

- Using a mechanical pressure gage

1. Ensure that the regulator's adjuster screw is almost entirely loosened (counter-clockwise). Check all tubing connections once again to make sure they are snug. Ensure that the ball valves on the manifold are turned off.
2. Open the shut-off valve at the wall by turning the handle to align with the direction of airflow.
3. Using the 5/16 inch wrench, tighten the adjuster screw (clockwise) while observing the gage. Tighten it to a desired 'high' pressure. A pressure of 26psi was confirmed to strain 2mm ID rings to 10% of their original diameter. It is recommended that the user recalibrate the pressure-diameter relationship of the silicone tubes if he/she desires accurate strain.

4. You may then close the shut-off valve once again.

- Using a pressure transducer

1. Connect an electronic display to the transducer.
2. Ensure that the regulator's adjuster screw is almost entirely loosened (counter-clockwise). Check all tubing connections once again to make sure they are snug. Ensure that the ball valves on the manifold are turned off.
3. Open the shut-off valve at the wall by turning the handle to align with the direction of airflow.
4. Using the 5/16 inch wrench, tighten the adjuster screw (clockwise) while observing the readout from the display. Tighten it to a desired 'high' pressure. A pressure of 26psi was confirmed to strain 2mm ID rings to 10% of their original diameter. It is recommended that the user recalibrate the pressure-diameter relationship of the silicone tubes if he/she desires accurate strain.
5. You may then close the shut off valve once again.

Low pressure and waveform calibration:

1. Screw empty 50mL media chambers onto each cap assembly and place inside the base stand holes.
2. Using a thin-tipped pipette, fill each silicone tube with water. Continue filling until even the bushing and connector are filled with water.
3. Connect the 4 inch LDPE tubes that were cut during assembly (see step #12 of "Connecting the manifold) to each of the 1/4 inch push-to-connect connectors.
4. Fill each of these tubes near completion with water.
5. Connect the remaining ends of the 4 inch LDPE tubes to the ball valves coming from the manifold.
6. Turn off the timer box.

7. Plug in the power cable and the solenoid valve into the timer box (each can only fit into one electrical outlet in the back of the box).
 8. Completely open all needle valves (counter-clockwise) using the small flathead screwdrivers.
 9. Open all ball valves.
 10. Set the timer to 0.5 seconds.
- For use with mechanical pressure gage:
11. Open the shut-off valve at the wall.
 12. Turn on the timer box.
 13. Observing the gage, you will notice that the pressure quickly increases and stops at 26 psi (or the value at which you calibrated the regulator) for a while until plunging downward. Slowly and continuously tighten the needle valve in front of the regulator while observing the upward motion of the gage needle. Eventually, you will see the needle take more time to reach the high pressure value. To critically dampen the upswing of the pressure wave, tighten the needle valve until it appears that the gage needle touches the high pressure value for an instant. This might take practice.
 14. Now observing the gage, you will notice that the pressure quickly decreases and settles near 0 psi before rising again. Turn several times clockwise the needle valve at the end of the air volume tank (the one open to the atmosphere) and pause for several cycles while observing the location at which the needle stops at its low end. A value of 4 psi was verified to strain the silicone tube to an OD of 2mm (it is recommended that the user recalibrate the pressure-diameter relationship of the silicone tubes if he/she desires accurate strain). Repeat this process of adjusting and observing until the needle reliably stops at a desired pressure at the wave's low end.
 15. To critically dampen the downswing of the pressure wave, tighten the needle valve between the air volume tank and the solenoid valve until it appears that the

gage needle touches the low pressure value for an instant. This might take practice.

16. Once these steps have taken place, the system might need to be fine-tuned.
17. With all components of the pressure system calibrated, you may turn off the timer and close the main shut-off valve.
18. Disconnect the LDPE tubes from all silicone tube assemblies and remove the conical media chambers.

- For use with pressure transducer:

11. Open the shut-off valve at the wall.
12. Turn on the timer box.
13. Connect the transducer to a software program (such as LabView) that enables you to view a pressure-time wave in real time.
14. Observing the wave on a real-time display, you will notice that the pressure quickly increases and stops at 26 psi (or the value at which you calibrated the regulator) for a while until plunging downward. Slowly and continuously tighten the needle valve in front of the regulator while observing the upward motion of the wave. Eventually, you will see the wave develop a smooth downward concave as it takes more time to reach the high pressure value. To critically dampen the upswing of the pressure wave, tighten the needle valve until it appears that the wave reaches the high pressure value for an instant. This might take practice.
15. Now observing the wave, you will notice that the pressure quickly decreases and settles near 0 psi before rising again. Turn several times clockwise the needle valve at the end of the air volume tank (the one open to the atmosphere) and pause for several cycles while observing the value at which the wave rests at its low end. A value of 4 psi was verified to strain the silicone tube to an OD of 2mm (it is recommended that the user recalibrate the pressure-diameter relationship of the silicone tubes if he/she desires accurate strain). Repeat this process of adjusting and observing until the wave reliably stops at a desired pressure at the its low end.

16. To critically dampen the downswing of the pressure wave, tighten the needle valve between the air volume tank and the solenoid valve until it appears that the wave touches the low pressure value for an instant. This might take practice.
17. Once these steps have taken place, the system might need to be fine-tuned.
18. With all components of the pressure system calibrated, you may turn off the timer and close the main shut-off valve.
19. Disconnect the LDPE tubes from all silicone tube assemblies and remove the conical media chambers.

Cleaning:

1. Wipe all materials with appropriate cloth and solution to minimize eventual contamination from skin oil, machine oil, and/or dust.

Autoclaving:

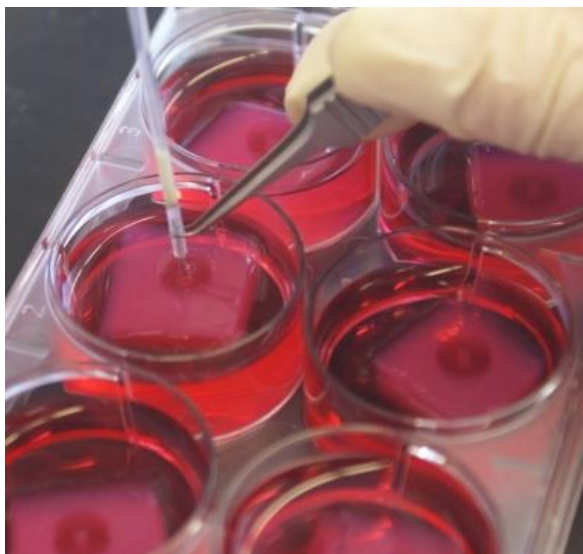
1. Using a thin-tipped pipette, fill each silicone tube assembly with water (lumen must be wet). You may simply use the water that remains in the silicone tubes from the pressure wave calibration.
2. Autoclave each cap/tube assembly.
 - a. For high-speed instrument (flash) sterilizer: 10 minutes at 132 degrees Celsius (270 degrees Fahrenheit) and 30 psi.
 - b. For standard gravity sterilizer: 30 minutes at 121 degrees celsius (250 degrees Fahrenheit) and 15 psi.

This information was found at <http://www.smimfg.com/tubing.html#clean>

Running the bioreactor

Ring Loading:

1. Prepare cell media in 8 separate 50mL conical tubes.
2. Once the cap assemblies have been sterilized, open the bag under a sterile hood using proper sterile technique.
3. Fit the outlet taper of a sterile air filter into the flexible silicone tube that protrudes from the side hole of each cap.
4. Bring the agar wells on which the cell rings remain underneath the sterile hood.
5. To load the cell rings, insert the end needle into the center post of the agar well. Ensure that the end of the silicone tube becomes flush with the top of the post and also that it is accurately centered. Refer to the picture below.



Inserting Needle Endcap Into Agarose Post

6. Using forceps, gently coax the ring up onto the silicone ring. There is no need to move a ring higher than about 1 inch past the silicone glue at the endplug.
7. Repeat step 5 for the same tube until 4 rings are loaded and spaced evenly. The lowest ring should not be closer than 0.25 inch from the top of the endplug's silicone glue.
8. Once rings are loaded onto a tube, gently stab the end needle into the PMMA center of an end weight using forceps.

9. Screw a media-filled chamber onto the cap assembly while careful not to bump the rings into the side of the chamber, and place it into a hole in the base stand.
10. Repeat steps 5 through 8 for the rest of the 8 tube assemblies.

Sterile tube assembly and startup:

1. Using a thin-tipped pipette, once again fill each silicone tube with water. The lumen does not have to be sterile although the outside of the tubes and the underside of the cap must be sterile. Continue filling until even the bushing and connector are filled with water.
2. Connect the 4 inch LDPE tubes that were cut during assembly (see step #12 of “Connecting the manifold) to each of the 1/4 inch push-to-connect connectors.
3. Fill each of these tubes near completion with water.
4. Connect the remaining ends of the 4 inch LDPE tubes to the ball valves coming from the manifold.
5. Place entire base and manifold assembly into incubator allowing the outlet tube of the solenoid valve to enter from the outside.
6. Open the shut-off valve at the wall.
7. Turn on the timer box.
8. The system is now running.

Changing the media:

1. Once media needs to be changed, close the two in-line ball valves of a single tube system (while the machine is still running). Closing more than this will significantly change the pressure capacitance of the bioreactor and will cause the low pressure of the wave to drop below what was calibrated.
2. Disconnect the target tube assembly by removing the ball valve closest to the chamber's cap from the tube that leads to the ball valve near the manifold. Do not disconnect the ball valve from the tube that leads directly to the silicone tube assembly (i.e. leave the LDPE tube that is filled with water connected to its ball valve).

3. Take that individual assembly under a sterile hood.
4. With sterile, fresh media prepared in a new 50mL conical chamber, remove the old chamber while taking care to not damage the rings, and screw the new chamber onto the cap.
5. Reconnect this assembly to the tube that was previously attached, and open first the ball valve near the manifold.
6. Open the ball valve near the silicone tube assembly.
7. Repeat steps 1 though 6 for each of the other tube assemblies in need of media replenishment.

Complete Parts List

Distribution Co.	Manufacturer	Item Number	Item Description	Price/piece
1 Specialty Manufacturing Inc	SMI	008490/000001	Silicone tubing tube 0.58" X 0.077" Durometer = 50 Clear length = 50ft	\$33.52
2 STC Valve	Sizto Tech Corp.	3S035-1/4-1	1/4" NPT Direct Acting 3 Way Stainless Steel Solenoid Valve (12VDC, DIN) 3.5mm orifice, viton seal	\$59.00
3 Minuteman Controls	Controlair	400-BA	General Purpose Regulator 0-30psi range, 1/4 NPT port size	\$47.25
4 Minuteman Controls	Legris	3140-56-00	1/4 OD Y union 10/box price is per piece	\$2.84
5 Minuteman Controls	Man-Aly	man-aly2-10	clip man-aly2-10 manifold 3/8 in input X 1/4 in output, 10 station	\$20.00
6 Minuteman Controls	Parker	218P-4	1/4 hex head plug	\$0.70
7 Minuteman Controls	Guest	PPSV040808W	1/4 OD POLYPROPYLENE SHUT-OFF VALVE, EPDM O-RING MUST BUY BAG QTY - 10 PER BAG Price is per piece	\$3.65
8 Minuteman Controls	MNV	MNV-3P	adj. control needle valve 1/8 NPT inlet, 10-32 outlet	\$6.03
9 Minuteman Controls	Legris	3175-56-14	Connector 1/4 OD X 1/4 NPT Male sold in box of ten	\$1.73
10 Minuteman Controls	Parker	209P-6-4	Bushing 3/8-1/4Bushing, pkg qty 25	\$1.00
11 Minuteman Controls	Guest	PPSV040808W	1/4 OD POLYPROPYLENE SHUT-OFF VALVE, EPDM O-RING MUST BUY BAG QTY - 10 PER BAG Price is per piece	\$3.65
12 Minuteman Controls	Parker	209P-4-2	1/4-1/8 Bushing, box qty is 25	\$0.64
13 Minuteman Controls	Legris	3171-56-20	1/4 OD X 10-32 UNF Male sold in boxes of 10 price is per piece	\$1.98
14 Minuteman Controls	Legris	3104-56-00	1/4 OD union tee sold in boxes of 10 price is per piece	\$2.84
15 Minuteman Controls	Memco	1/8B2-SS	1/8 Male Pipe thred to tube - stainless steel	\$3.90
16 Minuteman Controls	Parker	208P-4-2	Female-to-female, 1/4-1/8 reducer coupler	\$1.54
17 Minuteman Controls	Festo	CRVZS-2	2L Air volume tank	\$10.00
18 Minuteman Controls	Memco	1/8B1 S/S	1/8 Male Pipe thred to tube - stainless steel 304SS	\$3.51
19 Minuteman Controls	Legris	3102-56-00	1/4 OD Union Elbow sold in boxes of 10	\$2.70
20 Grainger	Grainger	6C758	Time Delay Relay	\$74.16
21 Row Inc.	Row, Inc.		O-ring, type - R, core - Silicone, encapsulation - FEP, ID 0.364in, CS 0.079in	\$6.32
22 Walgreens	BD	NDC/HR# 08290-8411-01; 328411	BD Ultra-Fine needle, bag of 10 sterile single use syringes for U 100 insulin	\$3.89
23 Radioshack			Detector Plug (2 per package)	\$2.99
24 The Home Depot			#7 O-ring	\$1.97
25 The Home Depot			9 piece wrench set	\$6.88
26 The Home Depot			8 piece wrench set	\$19.97
27 The Home Depot			1/4 NPT brass plug	\$2.33
28 The Home Depot		48643072169	Reducer	\$2.48
29 The Home Depot		48643072602	Pipe Bushing	\$2.72
30 Grainger		6RA24	Acorn nut, 4-40, pk5	\$8.35
31 Mouser Electronics	Mouser Electronics	602-FIT3501/8-25	Biocompatible heat sleeve 4ft long (Alpha wire FIT heat shrinkable tubing)	\$7.39
32 Omega	Omega	PX26 - 030GV	0-30 PSI pressure transducer	\$36.00
33 United States Plastic Corp.	USP Corp	58001	1/4" OD x .040 Wall Translucent Natural LDPE Tubing 100ft	\$11.44